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<p>(21) International Application Number: PCT/GB97/02284 (22) International Filing Date: 27 August 1997 (27.08.97) (30) Priority Data: 9618083.1 29 August 1996 (29.08.96) GB (71) Applicant (for all designated States except US): THE MINISTER OF AGRICULTURE FISHERIES & FOOD [GB/GB]; Whitehall Place, London SW1A 2HH (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): JARRETT, Paul [GB/GB]; 14 Home Furlong, Wellesbourne, Warwickshire CV35 9TW (GB). ELLIS, Deborah, June [GB/GB]; 7 Cooke Close, Warwick, Warwickshire CV34 5YG (GB). MORGAN, James, Alun, Wynne [GB/GB]; Pen-Y-Goruf Farm, Gorof Road, Ystradgynlais, Swansea SA9 1TP (GB). (74) Agent: SKELTON, S., R.; D/IPR, Formalities Section (Procurement Executive), Poplar 2, MOD Abbey Wood #19, P.O. Box 702, Bristol BS12 7DU (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>
<p>(54) Title: PESTICIDAL AGENTS</p> <p>(57) Abstract</p> <p>A method for killing pests (e.g. insects) comprising administering material from <i>Xenorhabdus</i> species (e.g. <i>X. nematophilus</i>) such as cells or supernatants orally to the pests, either alone or in conjunction with <i>Bacillus thuringiensis</i> or pesticidal materials derived therefrom. Also disclosed is an isolated pesticidal agent (and compositions comprising the same) characterised in that it is obtainable from cultures of <i>X. nematophilus</i> or mutants thereof, has oral pesticidal activity against <i>Pieris brassicae</i>, <i>Pieris rapae</i> and <i>Plutella xylostella</i>, is substantially heat stable to 55 °C, is proteinaceous, acts synergistically with <i>B. thuringiensis</i> cells as an oral pesticide and is substantially resistant to proteolysis by trypsin and proteinase K. DNA encoding pesticidal activity is also disclosed.</p>		

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PESTICIDAL AGENTS

The present invention relates to materials, agents and compositions having pesticidal activity which derive from bacteria, and more particularly from *Xenorhabdus* species. The invention further relates to organisms and methods employing such compounds and compositions.

There is an ongoing requirement for materials, agents, compositions and organisms having pesticidal activity, for instance for use in crop protection or insect-mediated disease control. Novel materials are required to overcome the problem of resistance to existing pesticides. Ideally such materials are cheap to produce, stable, have a high toxicity (either when used alone or in combination) and are effective when taken orally by the pest target. Thus any invention which provided materials, agents, compositions or organisms in which any of these properties was enhanced would represent a step forward in the art.

Xenorhabdus spp. in nature are frequently symbiotically associated with a nematode host, and it is known that this association may be used to control pest activity. For instance, it is known that certain *Xenorhabdus* spp. alone are capable of killing an insect host when injected into the host's hemocoel.

In addition, one extracellular insecticidal toxin from *Photorhabdus luminescens* has been isolated (this species was recently removed from the genus *Xenorhabdus*, and is closely related to the species therein). This toxin is not effective when ingested, but is highly toxic when injected into certain insect larvae (see Parasites and Pathogens of Insects Vol.2, Eds. Beckage, N. E. et al., Academic Press 1993).

Also known are certain low-molecular weight heterocyclic compounds from *P.luminescens* and *X.nematophilus* which have antibiotic properties when applied intravenously or topically (see Rhodes, S.H. et al., PCT WO 84/01775).

5

Unfortunately none of these prior art materials have the ideal pesticide characteristics discussed above, and in particular, they do not have toxic activity when administered orally.

10

The present invention provides pesticidal agents and compositions from *Xenorhabdus* species, organisms which produce such compounds and compositions, and methods which employ these agents, compositions and organisms, that alleviate some of the problems with the prior art.

15

According to one aspect of the present invention there is disclosed a method of killing or controlling insect pests comprising administering cells from *Xenorhabdus* species or pesticidal materials derived or obtainable therefrom, orally to the pests.

20

A PCT application of CSIRO published as WO 95/00647 discloses an apparently toxic protein from *Xenorhabdus nematophilus*; however no details of the protein's toxicity are given, and certainly there is no disclosure of its use as an oral insecticide.

25

Thus the invention provides an insecticidal composition adapted for oral administration to an insect, which composition comprises a pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these.

30

35

The composition may in fact comprise cells of *Xenorhabdus* or alternatively supernatant taken from cultures of cells of *Xenorhabdus* species. However, the composition

preferably comprises toxins isolable from *Xenorhabdus* as illustrated hereinafter. Toxic activity has been associated with material encoded by the nucleotide sequence of Figure 2. Thus, the composition suitably comprises a pesticidal material which is encoded by all or part of the nucleotide sequence of Figure 2. Pesticidal fragments as well as variants or derivatives of such toxins may also be employed.

The sequence of Figure 2 is of the order of 40kb in length. It is believed that this sequence may encode more than one protein, each of which may regulate or be insecticidal either alone or when presented together. It is a matter of routine to determine which parts are necessary or sufficient for insecticidal activity.

As used herein the term "variant" refers to toxins which have modified amino acid sequence but which share similar activity. Certain amino acids may be replaced with different amino acids without altering the nature of the activity in a significant way. The replacement may be by way of "conservative substitution" where an amino acid is replaced with an amino acid of broadly similar properties, or there may be some non-conservative substitutions. In general however, the variants will be at least 60% homologous to the native toxin, suitably at least 70% homologous and more preferably at least 90% homologous.

The term "derivative" relates to toxins which have been modified for example by chemical or biological methods.

These toxins are novel, and they and the nucleic acids which encode them form a further aspect of the invention.

A preferred *Xenorhabdus* species is the bacteria *X.nematophilus*. Particular strains of *X.nematophilus* which are useful in the context of the invention are

ATTC 19061 strain, available from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (NCIMB). In addition, suitable strains include two novel strains of *Xenorhabdus* which were deposited at the NCIMB
 5 on 10 July 1997 and were designated with repository numbers NCIMB 40886 and NCIMB 40887. These latter strains form a further aspect of the invention.

All strains have common characteristics as set out in the
 10 following Table 1.

Table 1

Strains

Characteristics	ATCC 19061	NCIMB 40887	NCIMB 40886
Gram strain	negative	negative	negative
Shape/size	rods up to 4µm long	rods up to 4µm long	rods up to 4µm long
Motile	Yes	Yes	Yes
Bioluminescent	No	No	No
Colour on NBTA*	blue	blue	blue
insecticidal on ingestion by insects	yes	yes	yes
Production of Antibiotics	yes	yes	yes
Resistant to ampicillin (50µg/ml)	yes	yes	yes
colony morphology/ colour	circular convex cream	circular convex cream	circular convex cream

15 *NBTA (Oxoid nutrient agar containing 0.0025%
 bromothymol blue and 0.004% tetrazolium chloride)

Preferably the pest target is an insect, and more preferably it is of the order Lepidoptera, particularly

Pieris brassicae, *Pieris rapae*, or *Plutella xylostella* or the order *Diptera*, particularly *Culex quinquefasciatus*.

In a preferred embodiment of the invention, cells from
5 *Xenorhabdus* species or agents derived therefrom are used in conjunction with *Bacillus thuringiensis* as an oral pesticide.

In further embodiments, rather than using *Bacillus*
10 *thuringiensis* itself, pesticidal materials obtainable from *B.thuringiensis* (e.g. delta endotoxins or other isolates) are used in conjunction with *Xenorhabdus* species.

15 The term 'obtainable from' is intended to embrace not only materials which have been isolated directly from the bacterium in question, but also those which have been subsequently cloned into and produced by other organisms.

20 Thus the unexpected discovery that bacteria of the genus *Xenorhabdus* (and materials derived therefrom) have pesticidal activity when ingested, and that such bacteria and materials can be used advantageously in conjunction with *B.thuringiensis* (and toxins or materials derived
25 therefrom), forms the basis of a further aspect of the present invention. The pesticidal activity of *B.thuringiensis* isolates alone have been well documented. However, synergistic pesticidal activity between such isolates and bacteria of the *Xenorhabdus* species (or
30 materials derived therefrom) has not previously been demonstrated.

In still further embodiments of the invention, culture supernatant taken from cultures of *Xenorhabdus* species,
35 particularly *X. nematophilus*, is used in place of cells from *Xenorhabdus* species in the methods above.

All of these methods can be employed, inter alia, in pest control.

The invention also makes available pesticidal
5 compositions comprising cells from *Xenorhabdus* species,
preferably *X.nematophilus*, in combination with *B.*
thuringiensis. As with the methods above, a pesticidal
toxin from *B.thuringiensis* (preferably a delta endotoxin)
may be used as an alternative to *B.thuringiensis* in the
10 compositions of the present invention

Likewise, culture supernatant taken from cultures of
Xenorhabdus species, preferably, *X.nematophilus* may be
used in place of cells from *Xenorhabdus* species.

15 Such compositions can be employed, inter alia, for crop
protection eg. by spraying crops, or for livestock
protection. In addition, compositions of the invention
may be used in vector control.

20 The invention further encompasses novel pesticidal agents
which can be isolated from *Xenorhabdus* spp. Techniques
for isolating such agents would be understood by the
skilled person.

25 In particular, such techniques include the separation and
identification of toxin proteins either at the protein
level or at the DNA level.

30 The applicants have cloned and partially sequenced a
region of DNA from *Xenorhabdus* NCIMB 40887 which region
codes for insecticidal activity and this is shown as
Figure 2 (SEQ ID NO. 1) hereinafter. Thus in a preferred
embodiment the invention also provides a toxin which is
35 encoded by DNA of SEQ ID No. 1 or a variant or fragment
thereof.

The invention also provides a recombinant DNA which encodes such a toxin. The recombinant DNA of the invention may comprise the sequence of Figure 2 or a variant or fragment thereof. Other DNA sequences may
5 encode similar proteins as a result of the degeneracy of the genetic code. All such sequences are encompassed by the invention.

The sequence provided herein is sufficient to allow
10 probes to be produced which can be used to identify and subsequently to extract DNA of toxin genes. This DNA may then be cloned into vectors and host cells as is understood in the art.

15 DNA which comprises or hybridises with the sequence of Figure 2 under stringent conditions forms a further aspect of the invention.

The expression "hybridises with" means that the
20 nucleotide sequence will anneal to all or part of the sequence of Figure 2 under stringent hybridisation conditions, for example those illustrated in "Molecular Cloning", A Laboratory Manual" by Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory Press, Cold Spring
25 Harbor, N.Y.

The length of the sequence used in any particular analytical technique will depend upon the nature of the technique, the degree of complementarity of the sequence,
30 the nature of the sequence and particularly the GC content of the probe or primer and the particular hybridisation conditions employed. Under high stringency, only sequences which are completely complementary will bind but under low stringency
35 conditions, sequences which are 60% homologous to the target sequence, more suitably 80% homologous, will bind. Both high and low stringency conditions are encompassed by the term "stringent conditions" used herein.

Suitable fragments of the DNA of Figure 2, i.e. those which encode pesticidal agents may be identified using standard techniques. For example, transposon
5 mutagenesis techniques may be used, for example as described by H.S. Siefert et al., Proc. Natl. Acad. Sci. USA, (1986) 83, 735-739. Vectors such as the cosmid CHRIM1, can be mutated using a variety of transposons and then screened for loss of insectidal activity. In this
10 way regions of DNA encoding proteins responsible for toxic activity can be identified.

For example, the mini-transposon mTn3(HIS3) can be introduced into a toxic *Xenorhabdus* clone such as CHRIM1,
15 hereinafter referred to as 'clone 1', by electroporating CHRIM1 DNA into *E.coli* RDP146(pLB101) and mating this strain with *E.coli* RDP146(pOX38), followed by *E. coli* NS2114Sm. The final strain will contain CHRIM1DNA with a single insertion of the transposon mTn3(HIS3). These
20 colonies can be cultured and tested for insecticidal activity as described in Example 8 hereinafter. Restriction mapping or DNA sequencing can be used to identify the insertion point of mTn3(HIS3) and hence the regions of DNA involved in toxicity. Similar approached
25 can be used with other transposons such as Tn5 and mTn5.

Site directed mutagenesis of CHRIM1 as outlined in "Molecular Cloning, A Laboratory Manual" by Maniatis, Fritsch and Sambrook, (1982) Cold Spring Harbor, can also
30 be used to test the importance of specific regions of DNA for toxic activity.

Alternatively, subcloning techniques can be used to identify regions of the cloned DNA which code for
35 insecticidal activity. In this method, specific smaller fragments of the DNA are subcloned and the activity determined. To do this, cosmid DNA can be cut with a suitable restriction enzyme and ligated into a compatible

restriction site on a plasmid vector, such as pUC19. The ligation mix can be transformed into *E. coli* and transformed clones selected using a selection marker such as antibiotic resistance, which is coded for on the
5 plasmid vector. Details of these techniques are described for example in Maniatis et al, supra, (see p390-391) and Methods in Molecular Biology, by L.G. Davies, M.D. Dibner and J.F. Battey, Elsevier, (see p222-224).

10 Individual colonies containing specific cloned fragments can be cultured and tested for activity as described in Example 8 hereinafter. Subclones with insecticidal activity can be further truncated using the same
15 methodology to further identify regions of the DNA coding for activity.

The invention also discloses an isolated pesticidal agent characterised in that the agent is obtainable from
20 cultures of *X. nematophilus* or variants thereof, has oral pesticidal activity against *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55°C, is proteinaceous, acts synergistically with *B.thuringiensis* cells as an oral pesticide and is
25 substantially resistant to proteolysis by trypsin and proteinase K.

By 'substantially heat stable to 55°C' is meant that the agent retains some pesticidal activity when tested after
30 heating the agent in suspension to 55°C for 10 minutes, and preferably retains at least 50% of the untreated activity.

By 'substantially resistant to proteolysis' is meant that
35 the agent retains some pesticidal activity when exposed to proteases at 30°C for 2 hours and preferably retains at least 50% of the untreated activity.

By 'acts synergistically' is meant that the activity of the combination of components is greater than one might expect from the use of the components individually. For example, when used in conjunction with *B.thuringiensis* cells as an oral pesticide, the concentration of *B. thuringiensis* cellular material necessary to give 50% mortality in a *P.brassicae* when used alone is reduced by at least 80% when it is used in combination the agent at a concentration sufficient to give 25% mortality when the agent is used alone.

It has been found that the activity of the material is retained by 30 kDa cut-off filters but is only partly retained by 100 kDa filters.

Preferably the agent is still further characterised in that the pesticidal activity is lost through treatment at 25°C with sodium dodecyl sulphate (SDS - 0.1% 60 mins) and acetone (50%, 60 mins).

Clearly the characterising properties of the isolated agent described above can be utilised to purify it from, or enrich its concentration in, *Xenorhabdus* species cells and culture medium supernatants. Methods of purifying proteins from heterogenous mixtures are well known in the art (eg. ammonium sulphate precipitation, proteolysis, ultrafiltration with known molecular weight cut-off filters, ion-exchange chromatography, gel filtration, etc.). The oral pesticidal activity provides a convenient method of assaying the level of agent after each stage, or in each sample of eluent. Such methodology does not require inventive endeavour by those skilled in the art.

The invention further discloses oral pesticidal compositions comprising one or more agents as described above. Such compositions preferably further comprise other pesticidal materials from non-*Xenorhabdus* species.

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These other materials may be chosen such as to have complementary properties to the agents described above, or act synergistically with it.

- 5 Preferably the oral pesticidal composition comprises one or more pesticidal agents as described above in combination with *B. thuringiensis* (or with a toxin derived therefrom, preferably endotoxin).
- 10 Recombinant DNA encoding said proteins also forms a further aspect of the invention. The DNA may be incorporated into an expression vector under the influence of suitable control elements such as promoters, enhancers, signal sequences etc. as is understood in the
- 15 art. These expression vectors form a further aspect of the invention. They may be used to transform a host organism so as to ensure that the organism produces the toxin.
- 20 The invention further makes available a host organism comprising a nucleotide sequence coding for a pesticial agent as described above.

Methods of cloning the sequence for a characterised

25 protein into a host organism are well known in the art. For instance the protein may be purified and sequenced: as activity is not required for sequencing, SDS gel electrophoresis followed by blotting of the gel may be used to purify the protein. The protein sequence can be

30 used to generate a nucleotide probe which can itself be used to identify suitable genomic fragments from a *Xenorhabdus* gene library. These fragments can then be inserted via a suitable vector into a host organism which can express the protein. The use of such general

35 methodology is routine and non-inventive to those skilled in the art. Such techniques may be applied to the production of *Xenorhabdus* toxins other than those encoded by the sequence of Figure 2.

It may be desirable to manipulate (eg. mutate) the agent by altering its gene sequence (and hence protein structure) such as to optimise its physical or
5 toxicological properties.

It may also be desirable for the host to be engineered or selected such that it also expresses other proteinaceous pesticidal materials (eg. delta- endotoxin from *B. thuringiensis*). Equally it may be desirable to generate
10 host organisms which express fusion proteins composed of the active portion of the agent plus these other toxicity enhancing materials.

15 A host may be selected for the purposes of generating large quantities of pesticidal materials for purification e.g. by using *B.thuringiensis* transformed with the agent-coding gene. Preferably however the host is a plant, which would thereby gain improved pest-resistance.
20 Suitable plant vectors, eg. the Ti plasmid from *Agrobacterium tumefaciens*, are well known in the art. Alternatively the host may be selected such as to be directly pathogenic to pests, eg. an insect baculovirus.

25 The teaching and scope of the present invention embraces all of these host organisms plus the agents, mutated agents or agent-fusion materials which they express.

Thus the invention makes available methods, compositions,
30 agents and organisms having industrially applicable pesticidal activity, being particularly suited to improved crop protection or insect-mediated disease control.

35 The methods, compositions and agents of the present invention will now be described, by way of illustration only, through reference to the following non-limiting examples and figures. Other embodiments falling within

th scope of the invention will occur to those skilled in
th art in the light of these.

FIGURE

- 5 Figure 1 shows the variation with time of the growth of
X. nematophilus ATCC 19061 and activity of cells and
supernatants against *P. brassicae* as described in Example
3.
- 10 Figure 2 shows the sequence of a major part of a cloned
toxin gene from *Xenorhabdus*.
- Figure 3 shows a comparison of the restriction maps of
cloned toxin genes from two strains of *Xenorhabdus*
15 (clone 1 above and clone 3 below).

EXAMPLES

20

Example 1 - Use of *X. nematophilus* cells as an oral
insecticide

- CELL GROWTH: A subculture of *X. nematophilus* (ATCC 19061,
25 Strain 9965 available from the National Collections of
Industrial and Marine Bacteria, Aberdeen, Scotland) was
used to inoculate 250 ml Erlenmeyer flasks each
containing 50 ml of Luria Broth containing 10g tryptone,
5g yeast extract and 5g NaCl per litre. Cultures were
30 grown in the flasks at 27°C for 40hrs on a rotary shaker.

- PRODUCTION OF CELL SUSPENSION: Cultures were centrifuged
at 5000 x g for 10 mins. The supernatants were discarded
and the cell pellets washed once and resuspended in an
35 equal volume of phosphate buffered saline (8g NaCl, 1.44g
Na₂HPO₄ and 0.24g of KH₂PO₄ per litre) at pH 7.4.

ACTIVITY OF CELL SUSPENSION TO INSECTS: The bioassays were as follows: *P. brassicae*: The larvae were allowed to feed on an artificial agar-based diet (as described by David and Gardiner (1965) London Nature, 207, 882-883) into which a series of dilutions of cell suspension had been incorporated. The bioassays were performed using a series of 5 doses with a minimum of 25 larvae per dose. Untreated and heat-treated (55°C for 10 minutes) cells were tested. Mortality was recorded after 2 and 4 days with the temperature maintained at 25°C.

Treatment	LC50 cells/g diet	
	2 days	4 days
Untreated	5.9×10^5	9.8×10^4
Treated 55°C	7.1×10^5	1.4×10^5

Aedes aegypti: The larva were exposed to a series of 5 different dilutions of cell suspension in deionised water. The biosassays were performed using 2 doses per dilution of 50 ml cell suspension in 9.5cm plastic cups with 25 second instar larvae per dose. Untreated and heat-treated (55°C or 80°C for 10 minutes) cells were tested. Mortality was recorded after 2 days with the temperature maintained at 25°C.

Treatment	LC50 cells/ml	
	2 days	
Untreated	5.1×10^6	
Treated 55°C	7.4×10^6	
Treated 80°C	$> 10^8$	

Culex quinquefasciatus: The larvae were exposed to a single concentration cell suspension containing 4×10^7 cells/ml. The biosassays were performed using 2 50 ml cell suspensions in 9.5 cm plastic cups with 25 second instar larvae per cup. Untreated and heat-treated (55°C or 80°C for 10 minutes) cells were tested. Mortality was

15

recorded after 2 days with the temperature maintained at 25°C.

	% Mortality
5 Treatment	2 days
Untreated	100
Treated 55°C	100
Treated 80°C	0

10 Thus these results clearly show that cells from *X. nematophilus* are effective as an oral insecticide against a number of insect species (and are particularly potent against *P. brassicae*). The insecticidal activity is not dependent on cell viability (i.e is largely unaffected by
15 heating to 55°C which reduces cell viability by >99.99%) but is much reduced by heating to 80°C, which denatures most proteins.

Example 2 - Use of *X. nematophilus* supernatant as an oral
20 insecticide

CELL GROWTH: Cultures were grown as in Example 1.

25 PRODUCTION OF SUPERNATANT: Cultures were centrifuged twice at 10000g for 10 mins. The cell pellets were discarded.

ACTIVITY OF SUPERNATANT TO INSECTS: The Bioassay was as follows:

30 Activity against neonate *P. brassicae* and two day old *Pieris rapae* and *Plutella xylostella* larvae was measured as for *P. brassicae* in Example 1, but using a series of untreated dilutions of supernatant in place of cell suspensions and with mortality being recorded after 4 days
35 only.

		LC50 (μ l supernatant/g diet)
Insect species		4 days
<i>P. brassicae</i>		22
5	<i>P. rapae</i>	79
	<i>P. xylostella</i>	135

In addition, size-reducing activity (62% reduction in 7 days) against *Mamestra brassicae* was detected in larvae fed on an artificial diet containing *X. nematophilus* supernatant (results not shown).

Thus these results clearly show that the supernatant from *X. nematophilus* culture medium is effective as an oral insecticide against a number of insect species, and are particularly potent against *P. brassicae*.

The heating of supernatants to 55°C for 10 minutes caused a partial loss of activity while 80°C caused complete loss of activity. Activity was also completely lost by treatment with SDS (0.1% w/v for 60 mins) and Acetone (50% v/v for 60 mins) but was unaffected by Triton X-100 (0.1% 60 mins), non-diet P40 (0.1% 60 mins), NaCl (1 M for 60 mins) or cold storage at 4°C or -20°C for 2 weeks. All of these properties are consistent with a proteinaceous agent.

The general mode of action of *X. nematophilus* cells and supernatants i.e. reduction in larval size and death within 2 days at high dosages, and other properties, eg. temperature resistance, appear to be similar suggesting a single agent or type of agent may be responsible for the oral insecticide activity activities of both cells and supernatants.

Example 3 - Timescale for appearance of ingestable insecticidal activity

CELL GROWTH: 1ml of an overnight culture of *X. nematophilus* was used to inoculate an Erlenmeyer flask. Cells were then cultured as in Example 1. Growth was estimated by measuring the optical density at 600 nm.

5

PRODUCTION OF CELL SUSPENSION AND SUPERNATANTS: These were produced as in Examples 1 and 2.

ACTIVITY OF CELLS AND SUPERNATANTS AGAINST *P. BRASSICAE*:

- 10 The cell suspension bioassay was carried out as in Example 1, but using a single dose of suspended cells equivalent to 50 μ l of broth/g diet and measuring mortality after 2 days. The cell supernatant bioassay was carried out as in Example 2, but using a single dose
15 equivalent to 50 μ l supernatant/g diet (i.e. more than twice the LC50) and measuring mortality after 2 days.

- The results are shown in Fig. 1. Thus these results clearly show that cells taken from *X. nematophilus*
20 culture medium are highly effective as an oral insecticide against *P. brassicae* after only 5 hours, and supernatants are highly effective after 20 hours. Although some slight cell lysis was observed in the early stages of growth, no significant cell lysis was observed
25 after this point demonstrating that the supernatant activity may be due to an authentic extracellular agent (as opposed to one released only after cell breakdown).

- Example 4 - Synergy between *X. nematophilus* cells and
30 *B. thuringiensis* powder preparations

- CELL GROWTH AND SUSPENSION: *X. nematophilus* cells were grown and suspended as in Example 1. *B. thuringiensis* strain HD1 (from *Bacillus* Genetic Stock Centre, The Ohio
35 State University, Columbus, Ohio 43210, USA) was cultured, harvested and formulated into a powder as described by Dulmage et al. (1970) J. Invertebrate Pathology 15, 15-20.

ACTIVITY OF *X. NEMATOPHILUS* CELLS AND *B. THURINGIENSIS* POWDER AGAINST *P. BRASSICAE*: The bioassays was carried out using *X. nematophilus* and *B. thuringiensis* in combination or using *B. thuringiensis* cell powder alone. Bioassays were carried out as in Example 1 but with various dilutions of *B. thuringiensis* powder in place of *X. nematophilus*. For the combination experiment, a constant dose of *X. nematophilus* cell suspension sufficient to give 25% mortality was also added to the diet. Mortality was recorded after 2 days.

		LC50 (μ g Bt powder/g diet)
<u>Bioassay</u>		<u>2 days</u>
15	B.t. alone	1.7
	B.t. plus <i>X.nematophilus</i>	0.09

These results clearly demonstrate the synergism between *X. nematophilus* cells and *B. thuringiensis* powder when acting as an oral insecticide against *P. brassicae*.

Example 5 - Synergy between of *X.nematophilus* supernatants and *B. thuringiensis* powder

CELL GROWTH AND PRODUCTION OF SUPERNATANTS: *X. nematophilus* cells were grown and supernatants prepared as in Example 2. *B. thuringiensis* was grown and treated as in Example 4.

ACTIVITY OF *X. NEMATOPHILUS* SUPERNATANTS AND Bt CELL POWDER AGAINST *P. BRASSICAE*: The bioassays were carried out using *X. nematophilus* supernatants and *B. thuringiensis* in combination or using *B. thuringiensis* powder alone. The Bioassay against neonate *P. brassicae* and two day old *Pieris rapae* and *Plutella xylostella* larvae were measured as in Example 2 but with various dilutions of *B. thuringiensis* in place of *X. nematophilus*. For the combination experiment, a

constant dose of *X. nematophilus* supernatant sufficient to give 25% mortality was also added to the diet. Mortality was recorded after 4 days.

5		LC ₅₀ (μ g Bt powder/g)	
	diet		
	<u>Insect species</u>	<u>Bt alone</u>	<u>Bt plus Xn</u>
	<i>P. brassicae</i>	1.4	0.12
	<i>P. rapae</i>	2.5	0.26
10	<i>P. xylostella</i>	7.2	0.63

These results clearly demonstrate the synergism between *X. nematophilus* supernatants and *B. thuringiensis* powder when acting as an oral insecticide against several insect species. The fact that both *X. nematophilus* cells and supernatants demonstrate this synergism strongly suggests that a single agent or type of agent is responsible for the demonstrated activities.

20 Example 5 - Characterisation of insecticidal agent from *X. nematophilus* supernatant by proteolysis

CELL GROWTH AND PRODUCTION OF SUPERNATANTS: *X. nematophilus* cells were grown and supernatants prepared as in Example 2.

PROTEOLYSIS OF SUPERNATANT: Culture supernatant (50ml) was dialysed against 0.5 M NaCl (3 x 1 l) for 48 hours at 4°C. The volume of the supernatant in the dialysis tube was reduced five-fold by covering with polyethylene glycol 8000 (Sigma chemicals). Samples were removed and treated with either trypsin (Sigma T8253 = 10,000 units/mg) or proteinase K (Sigma P0390 = 10 units/mg) at a concentration of 0.1 mg protease/ml sample for 2 hours at 30°C.

ACTIVITY OF PROTEASE TREATED SUPERNATANT AGAINST *P. BRASSICAE*: The bioassay against neonate *P. brassicae*

- larvae was carried out by spreading 25 μ l of each 'treatment' on the artificial agar-based diet referred to in Example 1 in a 4.5 cm diameter plastic pot. Four pots each containing 10 larvae were used for each treatment.
- 5 Mortalities were recorded after 1 and 2 days. Controls using water only, trypsin (0.1 mg/ml) and proteinase K (0.1 mg/ml) were also tested in the same way.

10 Treatment	% Mortality	
	1 day	2 days
Untreated supernatant	60	100
Proteinase K treated supernatant	45	100
Trypsin treated supernatant	40	100
All controls (no supernatant)	0	0

15

Example 6Entomocidal activity of other *Xenorhabdus*

- Using the methodology of Examples 1 and 2, four different
- 20 *xenorhabdus* strains were tested against insect pests.
- The results obtained were as follows:
- 1) Activity to *Pieris brassicae*

Strain deposit	Cells 10^6 /gram diet	Supernatant LC50
no/code	% mortality	μ l/gram of diet
NCIMB 40887	100	0.09
0014	100	0.52
0015	80	3.73
NCIMB 40886	100	0.05

- 25 It was found that entomocidal activity of cells and supernatant was reduced by more than 99% when all four strains were heated at 80°C for 10 minutes.

II) Activity to mosquitoes (*Aedes aegypti*)

Bacteria added at the rate of 10^7 cells/ml of water

Strain deposit no/code	Cells 10^6 /gram diet & mortality
NCIMB 40887	0
0014	40
0015	45
NCIMB 40886	95

- 5 Furthermore, all strains significantly reduced the growth of *Heliothis virescens*.

Example 7Cloning of toxin genes from strains of *Xenorhabdus*

- 10 Total cellular DNA was isolated from NCIMB 40887 and ATCC 19061 using a Quiagen genomic purification DNA kit. Cells were grown in L borth (10g tryptone, 5g yeast extract and 5g NaCl per l) at 28°C with shaking (150rpm) to an optical density of 1.5 A_{600} . Cultures were
- 15 harvested by centrifugation at 4000xg and resuspended in 3.5mls of buffer B1 (50mM Tris/HCl, 0.05% Tween 20, 0.5% Triton X-100, pH7.0) and incubated for 30 mins at 50°C. DNA was isolated from bacterial lysates using Quiagen 100/G tips as per manufacturers instructions. The
- 20 resulting purified DNA was stored at -20°C in TE buffer (10mM Tris, 1mM EDTA, pH 8.0).

- A representative DNA library was produced using total DNA of NCIMB 40887 and ATTC 19061 partially digested with the
- 25 restriction enzyme *Sau3a*. Approximately 20µg of DNA from each strain was incubated at 37°C with 0.25 units of the enzyme. At time intervals of 10, 20, 30, 45 and 60 minutes, samples were withdrawn and heated at 65°C for 15 minutes. To visualise the size of the DNA fragments, the
- 30 samples were electrophoresed on 0.5% w/v agarose gels.

The DNA samples which contained the highest proportion of 30 to 50kb fragments were combined and treated with 4 units of shrimp alkaline phosphatase (Boehringer) for 15 minutes at 37°C, followed by heat treatment at 65°C to
5 inactivate the phosphatase.

The size selected DNA fragments were ligated into the BamHI site of the cosmid vector SuperCos1 (Stratagene) and packaged into the *Escherichia coli* strain XL Blue 1,
10 using a Gigapack II packaging kit (Stratagene) in accordance with the manufacturers instructions.

To select for cosmid clones with entomocidal activity, individual colonies selected on L agar plates containing
15 25µg/ml ampicillin, were grown in L broth (containing 25µg/ml ampicillin) overnight at 28°C. Broth cultures (50µl) were individually spread onto the surface of insect diet contained in 4.5cm diameter pots, as described in Example 5. To each container 10 neonate *P. brassicae* larvae were added. Larvae were examined after
20 24, 72 and 96 hours recording mortality and size of surviving larvae. A total of 220 clones of NCIMB 40887 were tested, of which two were found to cause reduction in larval growth and death within 72 hours. Of 370
25 clones from ATTC 19061, one was found to cause larval death within 72 hours.

Example 8

Activity of cloned toxin genes to *Pieris brassicae*

30 The three active clones from Example 7 were grown in L broth, containing 25µg/ml ampicillin, for 24 hours at 28°C, on a rotary shaker at 150rpm. The activity of the toxin clones to neonate larvae were performed by incorporation of whole broth cultures into insect diet,
35 as described in Example 1.

<u>Clone No</u>	<u>Strain</u>	<u>LC50 (μl broth/g insect diet)</u>
1	NCIMB 40887	13.03
2	NCIMB 40887	16.7
3	ATTC 19061	108.7
Control*		No effect at 100 μ l/g

*XL1 Blue *E. coli* broth

5

When *E. coli* toxin clones were heated at 80°C for 10 minutes and added to the diet at a rate of 100 μ l/g, no activity to larvae was detected. Highlighting the heat sensitivity of the toxins.

10

Example 9

Sequencing of the cloned toxin from NCIMB 40887

Cosmid DNA of the entomocidal clone 1 above from NCIMB
15 40887 was purified using the Wizard Plus SV DNA system (Promega) in accordance with the manufacturers instructions. A partial map of the cloned fragment was obtained using a range of restriction enzymes *Eco*R1, *Bam*H1, *Hind*III, *Sal*I and *Sac*I as shown in Figure 3. DNA
20 sequencing was initiated from pUC18 and pUC19 based sub-clones of the cosmid, using the enzymes *Eco*R1, *Bam*H1, *Hind*III, *Eco*RV and *Pvu*II. Sequence gaps were filled using a primer walking approach on purified cosmid DNA. Sequence reactions were performed using the ABI PRISM™
25 Dye Terminator Cycle Sequencing Ready Reaction Kit with AmmpliTag DNA polymerase FS according to the manufacturers instructions. The samples were analysed on an ABI automated sequencer according to the manufacturers instructions. The major part of the DNA sequence for the
30 cloned toxin fragment is shown in Figure 2.

Example 10

Restriction map of cloned toxin from clone 3

Cosmid DNA of the entomocidal clone 3 above was purified
5 as described in Example 9. A restriction map of the
cloned fragment was obtained using the restriction
enzymes *Bam*H1, *Hind*III, *Sal*I and *Sac*I and this is shown
in Figure 3. When compared with the map from clone 1
(Figure 3) it is clear that over the regions which
10 overlap, the restriction maps are very similar. The
only detectable difference between the two clones was a
reduction in size of two *Hind*III fragments in clone 3,
corresponding to the 11.4kb and 7.2kb *Hind*III fragments
in clone 1 by approximately 2Kb and 200bp respectively.
15 These results indicate the overall relatedness of the DNA
region coding for toxicity in the two bacterial strains.

Example 11

Southern Blot Hybridisation Experiments

20 A 10.3kb *Bam*H1-*Sal*I fragment of the DNA from clone 1 was
used as a probe to hybridise to total *Hind*III digested DNA
of the *Xenorhabdus* strains ATCC 19061, NCIMB 40886 and
NCIMB 40887. Hybridisation was performed with 20ng/ml of
DIG labelled DNA probe at 65°C for 18 hours. Filters
25 were washed prior to immunological detection twice for 5
minutes with 2 x SSC (0.3M NaCl, 30mM sodium citrate, pH
7.0)/0.1% (w/v) sodium dodecyl sulphate at room
temperature, and twice for 15 minutes with 0.1 x SSC
(15mM NaCl, 1.5 mM sodium citrate, pH 7.0) plus 0.1%
30 sodium dodecyl sulphate at 65°C. The probe was labelled
and experiments performed in accordance with
manufacturers instructions, using a non-radioactive DIG
DNA labelling and detection kit (Boehringer). The probe
hybridised to a *Hind*III fragment of approximately 8kb in
35 all three strains as well as an 11.4kb fragment in NCIMB
40887 and an approximate 9kb fragment in both NCIMB 40886
and ATCC 19061. These results show that strains NCIMB

40886 and ATCC 19061 contain DNA with close homology to the toxin gene of clone 1 above, confirming the similarity between the toxins produced by the three strains.

5

CLAIMS

1. An insecticidal composition adapted for oral
5 administration to an insect comprising a pesticidal material obtainable from a *Xenorhabdus* species, or a pesticidal fragment thereof, or a pesticidal variant or derivative of either of these.
- 10 2. A composition according to claim 1 wherein the said pesticidal material comprises material encoded by the nucleotide sequence of Figure 2 or variant or fragment thereof, or a sequence which hybridises with said sequence.
- 15 3. A composition according to claim 1 or claim 2 which comprises cells of *Xenorhabdus*.
- 20 4. A composition as claimed in any one of the preceding claims which comprises supernatant taken from cultures of cells of *Xenorhabdus* species.
- 25 5. A composition according to any one of the preceding claims wherein the *Xenorhabdus* species is *Xenorhabdus nematophilus*.
- 30 6. A composition according to any one of claims 1 to 4 wherein the *Xenorhabdus* species is ATCC 19061, NCIMB 40886 or NCIMB 40887.
7. A composition as claimed in any one of the preceding claims which comprises a further pesticidal material not obtainable from *Xenorhabdus*.
- 35 8. A composition according to claim 7 wherein the said further pesticidal material comprises a material obtainable from *B. thuringiensis*.

9. A composition according to claim 8 which further comprises cells of *B. thuringiensis*.
10. A composition according to claim 8 wherein the
5 pesticidal materials obtainable from *B. thuringiensis* comprises the delta endotoxin.
11. A composition according to any one of the preceding claims which further comprises an agriculturally
10 acceptable carrier.
12. A composition according to claim 10 wherein the carrier comprises items of insect diet.
- 15 13. A method for killing or controlling insect pests, which method comprises administering to a pest or the environment thereof a composition according to any one of the preceding claims.
- 20 14. A method as claimed in claim 12 wherein the pests are insects from the order Lepidoptera or Diptera.
15. A microorganism comprising *Xenorhabdus* strain NCIMB 40886.
- 25 16. A microorganism comprising *Xenorhabdus* strain NCIMB 40887.
17. A pesticidal agent which comprises a a toxin
30 comprising a protein which is encoded by DNA which includes SEQ ID No. 1 or a variant or fragment thereof.
18. An isolated pesticidal agent characterised in that it is obtainable from cultures of *X. nematophilus* or
35 mutants thereof, has oral pesticidal activity against *Pieris brassicae*, *Pieris rapae* and *Plutella xylostella*, is substantially heat stable to 55°C, is proteinaceous, acts synergistically with *B. thuringiensis* cells as an

oral pesticide, and is substantially resistant to proteolysis by trypsin and proteinase K.

19. An isolated pesticidal agent as claimed in claim 18
5 further characterised in that the pesticidal activity is substantially destroyed by treatment with sodium dodecyl sulphate or acetone or heating to 80°C.

20. An isolated pesticidal agent as claimed in claim 18
10 or claim 19 further characterised in that the agent is an extracellular protein.

21. A recombinant DNA which encodes a pesticidal agent according to any one of claims 17 to 20.

15 22. A recombinant DNA of claim 21 which comprises the sequence of Figure 2 or a variant or fragment thereof.

23. A recombinant DNA which comprises or hybridises
20 under stringent conditions with all or part of the sequence of Figure 2, and which encodes a pesticidal material.

24. An expression vector comprising a recombinant DNA
25 according to any one of claims 21 to 23.

25. A host organism which has been transformed with an expression vector according to claim 24.

30 26. A host organism as claimed in claim 25 which has been engineered or selected such that it also expresses other pesticidal proteinaceous toxicity enhancing materials

27. A host organism comprising a nucleotide sequence
35 coding for a fusion protein comprising a pesticidally active portion of an agent as claimed in any one of claims 17 to 20 in combination with other pesticidal proteinaceous toxicity enhancing materials.

28. A host organism as claimed in claim 27 wherein the
pesticidal toxicity enhancing materials comprise delta-
endotoxin from *B. thuringiensis*.
- 5 29. A host organism as claimed in any one of claims 25 to
289 wherein the host is a plant.
30. A host organism as claimed in any one of claims 25 to
10 28 wherein the host is a virus pathogenic to insects.
31. A fusion protein as expressed by a host as claimed in
claim 27.
- 15 32. An pesticidal composition comprising one or more
agents as claimed in any one of claims 17 to 20.

Fig.1.

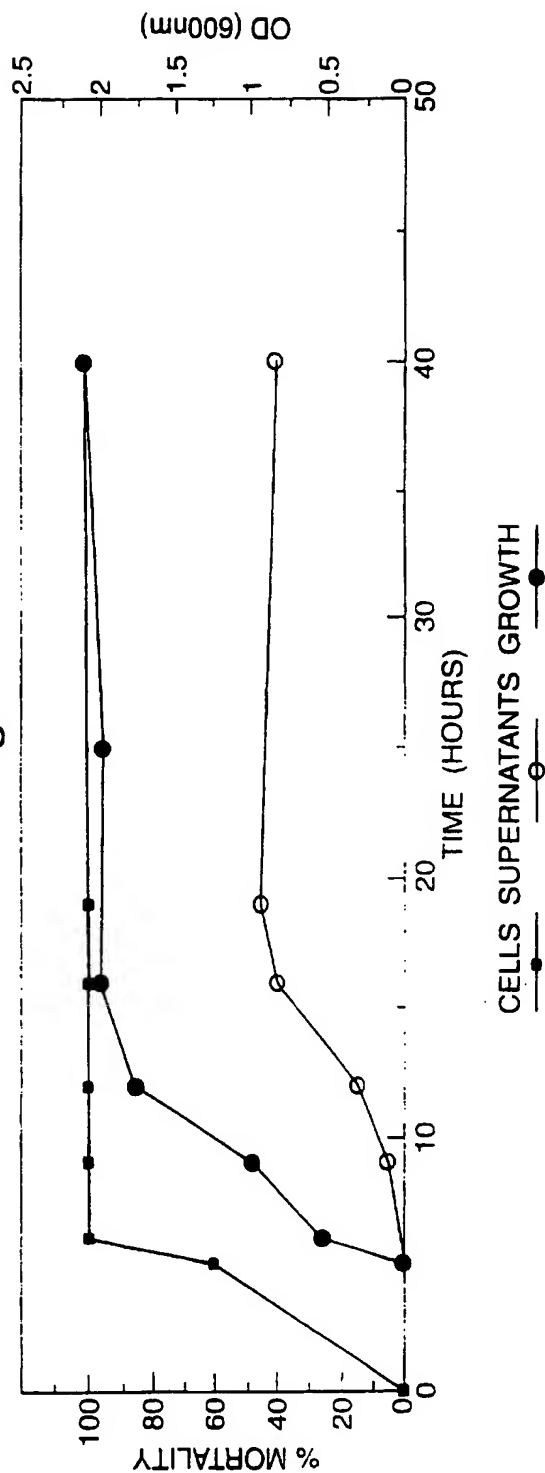


Fig.2.

1	TCCACAATTG	CCGGAGAAAA	TCAGTCGGGA	ACTGCCGGTG	ATTATTCTGTC	ACTTATTAAA
61	CGAATTGACC	GACCAGAATA	AGGCTAAAAA	ACTGCTACAG	GCGCAACGCG	ACTCGAACGA
121	AGCGTTAACG	GTAAAGAGTC	ATTCGGATCC	GCTGTATCGC	TTTTGTGGTT	ATCTGGTGTG
181	TGTCAATGAT	ATGACCGGAA	TGAAGATGGG	CAATAAAAAC	ATTAGCCAC	GAGCACCAG
241	ATTGTACTTG	TATCATGCCT	ATCTCTCTTT	TATGGAAGCG	CACGGCTTTG	AACGTCCGTT
301	AACACTGACT	AAGTTTGGTG	AATCCATCCC	CAAGATTATG	CTGGAATACC	GGAAAGGAGTA
361	TCGAAAAGTG	CGAACCAAGA	AAGGCTATTC	CTATAACGTG	GAATTATCGG	AAGAGGCCGA
421	AGAATGGCTA	CCGTCAGTGC	CTGAGTGTGC	AGACTTTAAA	TCACCTGTAT	AAAACTTTGA
481	GCTTTAAGTC	TGCACTCCAT	ACACAACCTA	AAATATCTAA	TTGTATTTAA	AAGAAAATAA
541	TAGATGTATA	GTTATTTTTT	AACTATACAT	AAGCTCTACA	TGCTCTTCAT	TCGTGTAAAA
601	AATGGGTGAA	CAGGTGATAC	AGTCAGTGAA	TATCATATTA	ATTACCGTAA	ACCCAGATGT
661	AGCAAGGCTT	TCAGGGAATT	GTGCAGAGGG	TGCATAACTG	AGAGGGTGCA	AAAGATTTC
721	AGGGGGGCTT	ATGGCAGGTA	AACAAAATCA	GAAGCAAATA	CCGTGCACAA	TCTGGTTTTT
781	ATTTTTTGGT	ACTACCTCAA	ATTAAAATGA	TGTAATCATC	TGATTTTTATT	TAAGAATAGA
841	AGTTAATCAC	AATTTTCATTG	ATGGACTTTC	ATTCACACTG	GTATAGATAA	ATAATTCTGT
901	TATATCCTGT	TTCAATTACG	ATTCATCAGG	AGTGTGTGTTA	CAGGAGACAA	GAATGTCTAC
961	CATCATTTAC	TTGTCGTTAA	AGGGCAAGAA	GCAGGGTTTA	ATTTACGCGG	GTTGTTCAC
1021	GCCTGAATCA	ATTGGAAATC	GCTATCAAAA	AGGACGTGAA	GATCAAATAC	AGGTATTGAG
1081	CCTGAATCAT	TCGATGAGCC	GTGACCAGAA	TGTTAATCAT	CAACCCGTCA	GTTTTGTGAA
1141	ACCCATTGAT	AAATCCTCTC	CCCTGTTTGC	TGGATGCCAG	TTTTGTGGCA	TACAGACAA
1201	CCGAGATGGG	ACAACTGGAG	TTCTTTTATG	AAATCAAGCT	GACCAGTGCC	ACGATTGTGG
1261	ATATTTCCCTA	TAATTATCCG	GCATTCAATC	AATGATAATG	GTGCGATACC	CCATGAAGTG
1321	GTGATGCTCG	ATTATAAGTC	CATTTTCATG	AACCAATCG	CCGCAGGACT	TCGGGCTACA
1381	GCATACGCAA	TTAGCCGGAA	TGGAAGAAGC	AAGCCGCTTT	TATCTGGGTT	CTCGAATGTT
1441	AAGCCACTTA	AGAAGCCGCT	GGTTGAAGAA	ACCCCGGTAA	AACCCGCTAA	ACATCATGCC
1501	CGTTATCGTT	GTGTGGATGA	TGACGGCAAT	CTTTTAACCG	AACGCAAGTA	TCGGGTTTGC
1561	CTGCCGGATG	GTGAGATAAA	AGAAGGAAAG	ACTGATAAAC	AAGGTTACAC	CCAATGGCAT
1621	CTTACGGATG	ACAAAAATAA	ACTTGAATTT	CATATTTTAA	AGGATTAATA	CCATGCCAGC
1681	CTATACCGTT	CAGACAAAAA	TAGAATCCAA	CGTACCTGTT	GAAAAACCTG	TTTACGACTT
1741	AACCATTTAT	CGTAAGGATG	CAAAAGGAAA	TTTCCATATC	TTGCTTGATG	TTTTTCAGGA
1801	GAAACTACAG	AGTAATTATG	AAACACAACA	GCATATCAGC	CAGGAAATAG	ACGACGATCT
1861	TTCTGTGATT	TATATTATGC	AAATTATGCT	TCACCGCAAA	CATGGCTCAA	ATATATTTCC
1921	GGCACTGCAA	ACCCATTTTA	AGAAAAATGA	TACCTCGGTT	GAATTAACCT	CCGGTAAAGC
1981	CTGTTCCGAG	AAAAAACGGG	AAAATGCCTG	TTATTTTGA	AGTACAGTTG	AAACAAAACC
2041	TGTCAGCGAC	GGGGATAATA	CCGTTGACTT	AAATATCACT	ATTCTCTGAC	GACCTTTTAT
2101	TGCCAAAGAA	TATCCCATTG	GTCAACCCACA	CGATCCATTT	GAAAAAAGTA	AAATTGAATC
2161	ATAAATACAG	GACAGGTTAT	CGAAAAGAAAT	TTATCCGGAT	CAAAATGGAG	CAAGTTTTATG
2221	TCAGGGCGCG	AGCACACTAT	TTTAGCTGCG	TTTTTAAAGAT	GATTATCTCT	TAATGTTTCAG
2281	TTTTAATAGT	GTTTTTATCG	AGTGAAATTT	AATCGCACAG	GCAATTCCTT	AGACTTTTAT
2341	AGAAAATAAA	AGAATTAAAG	AACAAGATTG	ACATTTTAA	TTCAAATATT	AATCAAAGTA
2401	TGCTCGCGCC	CTGAGTTTAT	GTGGCCCTGC	CGCTTTTTTT	TATTGCCTGC	CAATAGATAG
2461	ACCAGATATT	TATGAGCAAG	CGGCACGAGA	ATTATGGCAA	TATGGCCGAA	CTAAAATTGG
2521	TCAACTGGAA	ATTAAGCCGG	GTGAGGGTTG	CCGACATCCT	AAAGGTACTT	TTTATAATCA
2581	ATATGGTGAA	AGAATATCTG	GGTTAGATTG	GCTGACATTG	GCAAGCCTAA	GAGATTGAGA
2641	AAATATGATG	ATGAGGTTGA	TGATGAAGTA	GCTGGTATTA	CAATGTGGGG	AAAATTGACA
2701	GAATGGTTTG	AAAAATCAGG	GTATGAAAAA	GTATTTAGTA	ATGTCGGCTT	ATCCCATCT
2761	AATATAAATG	ACATAGTAAC	TCCTAGTGAT	TACTATAACA	AAGGATATCA	TGTGTGTTACT
2821	TTGATTTTCA	CAGGAATGTT	ATCAGATTTT	GGTGACATAG	AAACATCAGG	AAAAATCAT
2881	TGGATAGTTT	GGGAAGGAGT	AGTAGAAAAAC	TATGAGAAAG	AAAATATCAC	AAATAATTCA
2941	GATCTGAATC	AATATGTAAA	TTTAAATCTG	TTTTTCATGG	GTAAGTGGA	ACATCAAATT
3001	AAAAAAAACA	AATCACTAGA	TTATGTACTC	AACCATATTT	TTTGAGGGTT	GGTTTTTAAA
3061	CCAAATGAAAT	AACATGAAAA	AAATATTAAT	TATTTTTATT	TTTTTACTTT	ATGGTTGTGG
3121	TAATCCAACG	CCAAAAGTTT	TACCAAAATC	AGAGTTTCTT	CCTGATGCAG	TGATAAATGA
3181	ACCATATCAG	GCAATCAATTA	CCATCACAGG	AGGTGCATTG	AATGAAAAAA	GCCTTTGGGT
3241	AAAAATTTCAT	CCTACTGGCT	CAGGACTAAC	ATGGAATCCA	AAAGATAGTT	CTTTCCTATA
3301	GGGTGGAAAA	AAAGAAATAA	GAAAAGATTA	TCATCATATA	AATATAACAG	GTACCCCAAA
3361	GAAGACAGAA	TTGATAAAAA	TTGAAGTGGT	AGGATTTACA	TTGGGTACAA	TGTACGCAGC
3421	GAAAGAGTTC	ACTATAAATT	ATACTATAAA	AGTAAGGGAA	TAATTGTAC	TATCAGAAATG
3481	GTGATTTAAT	TCGCCATTTT	TATACTTTTG	TATACTCTCT	CAACATAATC	AGGATTCTTT

Fig.2.

3/12

3541	CTTATTATTT	TTCATGGTGC	TAAAAACGTT	TATTGCAAAA	ATAAAATTAAG	TTAATCAGAT
3601	AAATTATCTG	CATTACTGTT	ATAATCGATA	ACACGATAAC	CTGACTTTCT	GCCTGTTCTT
3661	ATGAACTCGA	AGATAATCCT	TTCTGAGCCT	GAACGAATCA	CATTGCAACC	ACTCGCTTTG
3721	AATCACCCAC	ACCGGGACAT	TCGTACGCGA	GGAACGGGTT	TACTCATGCT	TGCCAGAGGG
3781	AGCAAGCCGT	CCCAGATCAC	CGCTGAAATC	GGATGCAGTC	TCCGGGTAT	CTGTAATTGG
3841	GTTACATGT	GGCACAGATA	GCGGGATTAT	TCCGCGGTCA	TGCGGGAGGC	CGGTATCTCG
3901	CCATGACGCC	TGACATGATT	GCCACTGCGC	TGCAAGCCGC	CAGCGCAGAG	TCCCTGACGT
3961	CGCTCGAAGC	CAGGCAGGGT	TTCCCTGCCT	TGTACGCTTG	AAACGCTGGC	GAATACCCCTG
4021	AAAAAACAGG	GGCTCCCCCTA	TAAACGCCCC	CGCCTGTCGC	TTAAAAAAG	CGCAATAAAA
4081	CGGAGTTTGC	TGAAAAATCC	GCCTTGCTGA	ATAAAATTAA	GGCCGGAGCA	CAGTCAGGAC
4141	ATTACCGTCT	GGTCTATTTT	GAGTTCTGGG	GGCGTTAAAT	TACACGGATA	ACACGCTGTT
4201	TTACCAGACA	ACGTCAAGCA	GTATCACGCG	AGATGACGTG	ATTGATTTTT	TAGAGCCGGT
4261	GGCCAGACAA	GGGACAACCG	CCTGACATTT	TTAGTGTTGG	ATAATGCGCG	TATCCATCAC
4321	GGGATAGAGG	AAAAAATCAG	AAATGGCGGG	TGACGAGAAC	ACAACCTGTT	TTTATTCTAT
4381	CTTCCCCTT	ACAGCCCAGA	GCTGTATCTG	ATTGAAATCG	TCTGGAAACA	GGCCAAATAC
4441	GACTGGCGAC	GTTTATATC	CTGCACTCAG	GATACAATGG	AATATGAGGT	AAATACTTTA
4501	TTGAAAGGTT	ATGGCGACCA	ATTGCAATT	AACTTTTCTT	GAGTACTTAG	TAAGAATAGA
4561	GTCAGTCGAG	GTTTTTTCAT	TTCCGGTTCGT	GGGGATGATA	CTGAAAATTT	GTTTGTAATC
4621	TCTGAAAATT	GCTGTTTCTG	TGGCTACGTC	TGTCCTTTGG	GATATTGTTT	CCATCAAGTC
4681	TGTCAACATA	CTGTTAAGTT	AGATGTTGAT	AAAAGAGACT	GAATTATAAT	ACAAAACAAT
4741	AAATCACTTG	GACAATATTT	TATTTACAT	GAGACATTAA	GGTTGATTTT	CCCAATCTGG
4801	TCAGTTATAA	CCGAATAAGG	ATCTTGAAAA	ATCATGGGAT	CTTACTTTTA	TCAAATGAAG
4861	TTAACGTAAA	AGTTGATAAA	GAAAATTATT	TAATTCTAAG	TGCCGTTGGC	ATAAATATTT
4921	TGTGTTTTGT	TAATGAATGA	ATAACCAAGT	AAAGCTGGAT	TTTCAATTTT	ATTACTCGT
4981	TACAATATGC	TATTTATTTA	TATAAAGAGT	TTGTGCCCCA	TTAACCAGTA	AACAAATTTG
5041	TTCAACCGTA	ACTTAGCTTC	ATCGACTTTT	GGCCTCGCCT	GGTCAGAAATC	TAGGGCCGTT
5101	ATCCTATTTA	TTTATGATAA	ATAAAATTTA	ATTATCTTTA	ATAAGCTGAA	TATGTGGATT
5161	TGTGCTCAAT	CTTGGAATCA	AGTATGTATT	CCTTTTGTTA	CCCTGCTTTA	TTTTAAGGCA
5221	GATGAAGAGG	ATGCCAACAT	GACACAATAT	CGATTACGAC	TGTAACATTA	AAGTCAGTTA
5281	TAAATTTTAT	GATTAAAAATG	AAATTTTAGT	AGAAAATCGT	ATTCTATTCC	GCCATTTTACA
5341	ATAGCATCCT	CTTTAATATC	ATTAATCTCA	GATAAAACAA	ATAATTACAA	TGTGAATAGA
5401	ATAATGACTT	ACAAAATAAG	CACATAATCT	TCAGATGAAC	TCTTAACCTGA	CAACACTATT
5461	TTATAAAATA	ATTGAGGTTA	TTATGTATAG	CACGCTGTAT	TTACTCAATA	AAATCAGTCC
5521	CACCTCGGAC	GGTCAGACGA	TGACTCTTGC	GGATCTGCAA	TATTTATCCT	TCAGTGAATC
5581	GAGAAAAATC	TTTGATGACC	AGCTCAGTTG	GGGAGAGGCT	CGCCATCTCT	ATCATGAAAC
5641	TATAGAGCAG	AAAAAAAATA	ATCGCTTGCT	GGAAAGCGCT	ATTTTATCCC	GTGCCAACCC
5701	ACAATTATCC	GGTGCTATCC	GACTCGGTAT	TGAACGAGAC	AGCGTTTCAC	GCAGTTATGA
5761	TGAAATGTTT	GGTGCCCGTT	CTTCTTCCTT	TGTGAAACCG	GGTTCAGTGG	CTTCCATGTT
5821	TTCAACGGCT	GGCTATCTCA	CCGAATTGTA	TCGTGAAGCG	AAGGACTTAC	ATTTTTCAG
5881	CTCTGCTTAT	CATCTTGATA	ATCGCCGTCC	GGATCTGGCT	GATCTGACTC	TGAGCCAGAG
5941	TAATATGGAT	ACAGAAATTT	CCACCCTGAC	ACTGTCTAAC	GAACTGTTGC	TGGAGCTATT
6001	ACCCGCAAGA	CCGGAGGTGA	TTCCGACGCA	TTGATGGAGA	GCCTGTCAAC	TTACCGTCAG
6061	GCCATTGATA	CCCCTTACCA	TCAGCCTTAC	GAGACTATCC	GTCAGGTATC	TATGACCCAT
6121	GACAGTACAC	TGTCAGCGCT	GTCCCCTAAT	CCTGAGGTGA	TGGGGCAGGC	GGAAAGGGCT
6181	TCATTACTGG	CGATTCTGGC	CAATATTTCT	CCAGAACTGT	ATAACATTTT	GACCGAAGAG
6241	ATTACGGAAA	AGAAACGCTGA	TGCTTTATTT	GCGCAAAACT	TCAGTGAAAA	TATCACGCCC
6301	GAAAAATTCG	CGTCACAATC	ATGGATAGCC	AAGTATTATG	GTCTTGAATC	TTCTGAGGTG
6361	CAAAAATACC	TCCGGATGTT	GCAGAATGGC	TATTCTGACA	GCACCTCTGC	TTATGTGGAT
6421	AATATCTCAA	CGGGTTTAGT	GGTCAATAAT	GAAAGTAAAC	TCGAAGCTTA	CAAAAATAACA
6481	CGTGTA AAAA	CAGATGATTA	TGATAAACAT	GTAATTTACT	TTGATCTGAT	GTATGAAGGA
6541	AATAATCAAT	TCTTTATATG	TGCTAATTTT	AAGATATCGA	GAGAAATTTG	GGCGACTCTT
6601	AGGAAAAACT	CAGGGACAAG	TGGCATGTGC	GGCAGCCTTT	CCGGTCCCCCT	GGTAGCCAAT
6661	ACTAATTTCA	AAAGCAATTA	CTTAAGTAAC	ATATCTGATA	ATGAATACAG	AAATGGCGTA
6721	AAAATATATG	CCTATCGCTA	TACGTCTTCC	ACCAGCGCCA	CAAATCAGGG	CGGCGGAATA
6781	TTCACTTTTG	AGTCTTATCC	CCTGACTATA	TTTGCCTCA	AACTGAATAA	AGCCATTTCG
6841	TTGTGCCTGA	CTAGCGGGCT	TTACCGGAAT	GAACTGCAAA	CTATCGTAGC	CAGTGACAAT
6901	GCACAAGGCA	TCATCAACGA	CTCCGTCTCG	ACCAAAGTTT	TCTATACTCT	GTTCTACAGT
6961	CACCGTTATG	CACTGAGCTT	TGATGATGCA	CAGGTACTGA	ACGGATCGGT	CATTAAATCAA
7021	TATGCCCGAC	GATGACAGTG	TCAGTCATTT	TAACCGTCTC	TTTAATACCC	CGCCGCTGAA
7081	AGGGAAAATC	TTTGAAGCCG	ACGGCAACAC	GGTCAGCAAT	GATCCGGATG	AAGAACAAATC
7141	TACCTTTGCC	CGTTACGCCC	TGATGCGTGG	TCTGGGGATC	AACAGTGGTG	AACTGTATCA
7201	GTTAGGCAAA	CTGGCGGGTG	TATTGGACAC	ACAAAATATC	CTCACACTTT	CTGTCCCTGT
7261	TATATCTTCA	CTGTATCGCC	TCACGTTACT	GGCCCGTGCC	CATCAGCTGA	CGGTTAATGA
7321	ACTGTGTATG	CTTTATGGTT	TTTCGCCGTT	CAATGGCAAA	ACAACGGCTT	CTTTGTCTTC

Fig.2.

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7381	CGGGGAGTTG	TCACGGCTGG	TTATCTGGTT	GTATCAGGTG	ACGCAGTGGC	TGACTGAGGG
7441	CGGAAATCAC	CACTGAAGCG	ATCTGGTTAT	TATGTACGCC	AGAGTTCAGC	GGGAATATTT
7501	CACCGGAAAT	CAGTAATCTG	CTTAATACTC	TCCGACCCCG	TATTAGTGAA	GACATGGCAC
7561	AAAGTAGTGA	CCGGGAGCTT	CAGGCTGAAA	TTCTCGCGCC	GTTTATTGCT	GCAACCGCTGC
7621	ATCTGGCGTC	ACCAGATATG	GCGCGGTATA	TCCTGTTGTG	GACTGATAAC	CTGCGGCCGG
7681	GCGGCCTGAA	TATCGCCGGA	TTTATGATGC	TGGTGCTGAA	AGAGACGCTG	AGTGATGAGG
7741	AAACGACCCA	ACTGGTTCAA	TTCTGCCATG	TAATGGCACA	GTTATCGCTT	TCCGTGCAGA
7801	CACTGCGTCT	CAGTGAAGCA	GAGCTTCTG	TGCTGGTCAT	TTCCGATTTT	GTGGTACTGG
7861	GTGCGAGAAG	CCAACCGCCG	GACAACACAA	TATTGATACT	CTGTTCTCAC	TCTACCGATT
7921	CCACCACTGG	ATTAATGGGC	TGGGAAATCC	CGGCTCTGAC	ACGCTGGATA	TGCTGCGCCA
7981	AGCAGACACT	CACGGGCGAC	AGACTGGGCC	TCCGTGATGG	GGCTGGACAT	CAGTATGGTA
8041	ACGCAGGCCA	TGGGTTCCCG	CCGGCGTGAA	CCAACCTTCAG	TGTTGGCAGG	ATATCAACCC
8101	CGTGTTGCAG	TGGATACATG	TGGCATCAGC	ACTGCTCACT	GATGCCGTGC	GTTATCCGTA
8161	CCCTGGTGAA	TATCCGTTAC	GTGACTGCAT	TAAACAAAGC	CGAGTCGAAT	CTGCCCTGCC
8221	GGGATAAGTG	GCAGACGCTG	GCAGAAAATA	TGGCAGCCGG	ACTGAGTACA	CAACAGGCTC
8281	AGACGCTGGC	GGATTATACC	GCAGAGCGCC	TGAGTAACGT	GTTGTGCAAT	TGGTTTCTGG
8341	CGAATATCCA	GCCAGAAGGG	GTGTCCCTGC	ACAGCCGGGA	TGACCTGTAC	AGCTATTTCC
8401	TGATTGATAA	TCAGGTCTCT	TCTGCCATAA	AAACCACCCG	ACTGGCAGAG	GCCATTGCCG
8461	GTATTACAGT	CTACATCAAC	CGGGCGCTGA	ACCGGATAGA	GCCTAATGCC	CGTGCCGATG
8521	TGTCAACCCG	CCAGTTTTTT	ACCGACTGGA	CGGTGAATAA	CCGTTACAGC	ACCTGGGGCG
8581	GGGTGTCGCG	GCTGGTTTTAT	TATCCGGAAA	ATTACATTGA	CCCGACCCAG	CGTATCGGGC
8641	AGACCCGGAT	GATGGATGAA	CTGCTGGAAG	ATATCAGCCA	GAGTCAGCTC	AGCCGGGACA
8701	CGGTGGAAGA	GGCCTTTAAA	ACTTACCTGA	CCGCTTTGAA	ACCGTGGCAG	ACCTGAAAGT
8761	TGTCAGCGCT	ATCACCGACA	ACGTCAACAG	CAACACCGGA	CTGACCTGGT	TTGTCGGCCA
8821	AACGCGGGAG	AACCTGCCGG	AATATTACTG	GCGTAACGTG	CATATATCAC	GGATGCAGGC
8881	GGGTGAAC TG	GCCGCCGATG	CCTGGAAAGA	TTGGACGAAG	ATTGATACAG	CGGTCAACCC
8941	ATACAAGGAT	GCAATACGTC	CGGTCAATAT	CAGGGAACGT	TTGCACCTTA	TCGTGGGTAG
9001	AAAAAGAGGA	AGTGGCGAAA	AATGGTACTG	ATCCGGTGGA	AACCTATGAC	CGTTTTACTC
9061	TGAAACTGGC	GTTTTCTGCT	CATGATGGCA	GTGGAGTGC	CCCTGGTCT	TACGATATCA
9121	CAACGCAAGT	GGAGGCCGTC	ACTGACAAAA	AACCTGACAC	TGAACGGCTG	GCGCTGGCCG
9181	CATCAGGCTT	TCAGGGCGAG	GATACTCTGC	TGGTGTGTTG	GTACAAAACC	GGGGTGAGTT
9241	ACC CGGATTT	TGGCGACAAC	AATAAAAATG	TGGCAGGCAT	GACCATTTAC	GGCGATGGCT
9301	CCTTCAAAAA	GATGGAGAAC	ACAGCACTCA	GCGTTACAGC	CAACTGAAAA	ATACCTTTGA
9361	TATCATTCAAT	ACTCAAGGCA	ACGACTTGGT	AAGAAAGGCC	AGCTATCGTT	TCGCGCAGGA
9421	TTTTGAAGTG	CCTGCCCTCG	TGAATATGGG	TTCTGCCATC	GGTGATGATA	GTCTGACGGT
9481	GATGGAAAAC	GGGAATATTTC	CGCAGATAAC	CAGTAAATAC	TCCAGCGATA	ACCTTGCTAT
9541	TACGCTACAT	AACGCCGCTT	TCAGTGTGTC	ATATGATGGC	AGTGGCAATG	TCATCAGAAA
9601	CAAAACAAATC	AGCGCCATGA	AACTGACGGG	GTTGGATGAA	AGTCCCAGTA	CGGCAATGCA
9661	TTTATCATCG	CAAATACCGT	TAAACATTAT	GGCGGTTACT	CTGATCTGGG	GGGCCCGATC
9721	ACCGTTTTTA	TTAAAACGGA	AAAACATAT	TGCATCAGTT	CAAGGCCACT	TGATGAACGC
9781	AGATTACACT	AGGCGTTTGA	TTCTAACACC	AGTTGAAAAT	AATTATTATG	CCAGATTGTT
9841	CGAGTTTCCA	TTTTCTCCAA	ACACAATTTT	AAACACCGTT	TTACGCGTTG	GTAGCAATAA
9901	AACCACTGAT	TTTAAAAAGT	GCAGTTATGC	TGTTGATGGT	AATAATTCTC	AGGGCTTCCA
9961	GATATTTAGT	TCCTATCAAT	CATCCGGCTG	GCTGGATATT	GACACAGGTA	TTAACAAATC
10021	TGATGTCAAA	ATTACGGTGG	TAGCTGGCAG	TAAAACCCAC	ACCTTTACGG	CCAGTGACCA
10081	TATTGCTTCC	TTGCCGGCAA	ACAGTTTTGA	TGCTATGCCG	TACACCTTTA	AGCCACTGGA
10141	AATCGATGCT	TCATCGTTGG	CCTTTACCAA	TAATATTGCT	CCTCTGGATA	TCGTTTTTGA
10201	GACCAAAGCC	AAAGACGGGC	GAGTGCTGGG	TAAGATCAAG	CAAACATTAT	CGGTGAAACG
10261	GGTAAATTAT	AATCCGGAAG	ATATTCTGTT	TCTGCGTGAA	ACTCATTCCG	GTGCCCAATA
10321	TATGCAGCTC	GGGGTGTATC	GTATTCTGCT	TAATACCCTG	CTGGCTTCTC	AACCTGGTATC
10381	CAGAGCAAAC	ACGGGCATTG	ATACTATCCT	GACAATGGAA	ACCCAGCGGT	TACCGGAACC
10441	TCCGTTGGGA	GAAGGCTTCT	TTGCCAACTT	TGTTCTGCCT	AAATATGACC	CTGCTGAACA
10501	TGGCGATGAG	CGGTGGTTTA	AAATCCATAT	CGGGAATGTT	GGCGGTAACA	CGGGAAGGCA
10561	GCCTTATTAC	AGCGGAATGT	TATCCGATAC	GTCGGAACCC	AGTATGACAC	TGTTTGTCCC
10621	TTATGCCGAA	GGGTATTACA	TGCATGAAGG	TGTCAGATTG	GGGGTTGGAT	ACCAGAAAAAT
10681	TACCTATGAC	AACACTTGGG	AACTCTGCTT	CTTTTATTTT	GATGAGACAA	AACAGCAATT
10741	TGTATTAATT	AACGATGCTG	ATCATGATTC	AGGAATGACG	CAACAGGGGA	TCGTGAAAAA
10801	TATCAAGAAA	TACAAAAGGAT	TTTGTGAATG	TTCTATCGCA	ACGGGCTATT	CCGCCCCGAT
10861	GGATTTCAAT	AGTGCCAGCG	CCCTCTATTA	CTGGGAATGT	TCTATTACAC	CCCGATGATG
10921	TGCTTCCAGC	GTTTGCTACA	GGAAAAACAA	TTCCGACGAAG	CCACACAATG	GATAAACTAC
10981	GTCTATAATC	CCGCCGCTA	TATCGTTAAC	GGAGAAATCG	CCCCCTGGAT	CTGGAATGCG
11041	CGGCCGCTGG	AAGAGACACT	CCTGGAATGC	CAATCCGTTG	GATGCCATTG	ATCCGGATGC
11101	CGTCGCACAA	TATGACCGCA	CACACTATAA	AGTTGCCACC	TTTATGCGCC	TGTTGGATCA
11161	ACTTATTCTG	CGCGGCGATA	TGSCCTATCG	CGAACTGACC	CGCGATGCGT	TGAATGAAGC

Fig.2.

11221	CAAGATGTGG	TATGTGCGTG	CTTTGGAATT	GCTGGGTGAT	GAGCCGGAGG	ATTACGGCAG
11281	CCAACAGTGG	GCCGCACCGT	CTCTTTCCGT	GGCGGGCAAC	CACACTGTGC	AAGCGGGCTA
11341	TCAACAAGAC	CTTACGGCGC	TAGACAACGG	AGAAGGTTGC	ACTCAACCCC	GCAACGCTAA
11401	CTCGTTGGTG	GTTTGGTCCT	GCCGGAATAT	AACCCGGAAT	CAACCGATTA	CTGGCAAACC
11461	TGCGTTTGCG	CCTGGTTAAC	CTGCGCCATA	ATCCTTCCAT	GACGGGCAAC	CGTTATCGCT
11521	GGCGAATTAC	GCGAGCCTAC	GATCCGAAAG	CGCTGCTCAC	CAGTATGGTA	CAGCCTTCTC
11581	AGGGCGGTAG	TGCAGTGTCT	CCCGGCACAT	TGTCGTTATA	CCGCTTCCCG	GTGATGCTGG
11641	AGCGGGCCCG	CAATCTGGTA	GCGCAATTAA	CCCAGTTCGG	CACCTCTCTG	CTCAGTATGG
11701	CAGAGCATGA	TGATGCCGAT	GAATCACCAC	CGTTGCTACT	ACAGCAGGGT	ATGGAACCTG
11761	CGACACAGAG	CATCCGTATT	CAGCAACGAA	CTGTCGATGA	AGTGGATGCT	GATATTGCTG
11821	TATTGGCAGA	GAGCCGCCGC	AGTGACACAA	ATCGTCTGGA	AAAATACCAG	CAGCTGTATG
11881	ACGAGGATAT	CAACCACGGA	GAACAGCGTG	CGATGTCACT	GTTTGATGCG	GCGCGAGGTC
11941	AGTCTCTGGC	CGGGCAGGCG	CTCTCAGTAG	CAGAAGGGGT	GGCTGACTTA	GTTCCAAACG
12001	TGTTCCGTTT	CGCTTGTGGC	GGCAGTCGTT	GGGGGGCAGC	ACTGCGTGCT	TCCGCCTCCG
12061	TGATGTGCGT	TTCTGCCACA	GCTTCCCAAT	ATTCCGCAGA	CAAAATCAGC	CGTTCGGAAG
12121	CCTACCGCCG	CCGCCGTCAG	GAGTGGGAAA	TTTCCGCTGA	TAATGCTGAC	GGTGAAGTCA
12181	AACAAATGGA	TGCCCAGCTG	GAAAGCCTGA	AAATACGCGG	CGAAGCAGCA	CAGATGCAGG
12241	TGGAATATCA	GGAGACCCAG	CAGGCCCATATA	CTCAGGCTCA	GTTAGAGCTG	TTACAGCGTA
12301	AATTACACAA	CAAAGCGCTT	TACAGTTGGA	TGCGCGGCAA	GCTGAGTGCT	ATCTATTACC
12361	AGTTCTTTGA	CCTGACCCAG	TCCTTCTGCC	TGATGGCACA	GGAAAGCTCG	GCGCGAGGTC
12421	TGACCGACAA	CGGTGTTACC	TTTATCCGGG	GTGGGGCCTG	GAACGGTACG	ACTGCGGGTT
12481	TGATGGCGGG	TGAAACGTTG	CTGCTGAATC	TGGCAGAAAT	GGAAAAAGTC	TGGCTGGAGC
12541	GTGATGAGCG	GGCACTGGAA	GTGACCCGTA	CCGTCTCGTT	GGCACAGTTG	TATCAGGCCCT
12601	TATCATCAGA	CAACTTTAAT	CTGACCGAAA	AACTCACGCA	ATTCCTCGCT	GAAGGGAAAG
12661	GCAACGTAGG	AGCTTCCGGC	AATGAATTAA	AACTCAGTAA	CCGCCAGATA	GAAGCCTCAG
12721	TGCGATTGTC	TGATTTGAAA	ATTTTCAGCG	ATACCCCGGA	AAGCTTTGGC	AATACCCGTC
12781	AGTTGAAACA	AGTGAGTGTC	ACCTTGCCGG	CGCTGGTTGG	TCCGTATGAA	GATATCCGGG
12841	CGGTGCTGAA	TTACGGCGGC	AGCATCGTCA	TGCCACGCGG	TTGCACTGCT	ATTGCTCTCT
12901	CCACCGGCGT	GAATGACAGT	GGTCAATTTA	TGCTGGATTT	CAACGATTCC	CGTTATCTGC
12961	CGTTTGAAGG	TATTTCCGTG	AATGACAGCG	GTAGCCTGAC	GTTGAGTTTC	CCGGATGCGA
13021	CTGATCGACA	GAAAGCGCTG	CTGGAGAGCC	TGAGCGATAT	CATTCTGCAT	ATCCGCTATA
13081	CCATTGCTTC	TTAATTAAAA	CATTGTGATA	GGCAGGCTCC	TGAGGGAGCC	TGTTAAAGGA
13141	GTTTTTATGC	AGGGTTCAAC	ACCTTTGAAA	CTTGAAATAC	CGTCATTGCC	CTCTGGGGGC
13201	GGATCACTAA	AAGGAATGGG	AGAAGCACTC	AATGCCGTCG	GAGCGGAAGG	GGAGCGTCAAT
13261	TTTCACTGCC	CTTGCCGATC	TCTGTCCGGC	GTGGTCTGGT	GCCGGTGCTA	TCACTGAATT
13321	ACAGCAGTAC	TGCTGGCAAT	GGTTCATTCC	GGATGGGGTG	GCAATGTGGG	GTGGGTTTAA
13381	TCAGCCTGCG	TACCGCCCAAG	GGCGTTCCGC	ACTATACGGG	ACAAGATGAG	TATCTCGGGC
13441	CGGATGGGGA	AGTGTGAGT	ATTGTGCCGG	ACAGCCAAGG	GCAACCAGAG	CAACGCACCG
13501	CAACCTCACT	GTTGGGGACG	GTCTGACAC	AGCCGCCTAC	TGTTACCCGC	TATCAGTCCC
13561	GCGTGGCAGA	AAAAATCGTT	CGTTTAGAAC	ACTGGCAGCC	ACAGCAGAGA	CGTGAGGAAG
13621	AGACGTCCTT	TTGGGTACTT	TTTACTGCGG	ATGGTTTAGT	GCACCTATTC	GGTAAGCATC
13681	ATCATGCACG	TATTGCTGAC	CCGCAGGATG	AAACCAGAAAT	TGCCCGCTGG	CTGATGGAGG
13741	AAACCGTCA	GCATACCGGG	GAACATATTT	ACTATCACTA	TCGGGCAGAA	GACGATCTTG
13801	ACTGTGATGA	GCATGAACCT	GCTCAGCATT	CAGGTGTTAC	GGCCCAACCT	TATCCTGGCA
13861	AGTCCACTAT	GGCAATACTC	AGCCGGAAAC	CGCTTTTTC	GCGGTAAAT	CAGGTATCCC
13921	TGTTGATAAT	GAATGCTGTT	TTTACTGCGG	ATTTGATTAC	GGTGAGCGCT	TATCTTCGCT
13981	GAACTCCGTA	CCCGAATTCA	ATGTGTGAGA	AAACAATGTG	TCTGAAACCA	ATGTGTCTGA
14041	AAAATGGCGT	TGTCGTCCGG	ACAGTTTCTC	CCGCTATGAA	TATGGGTTTG	AAATTCGAAC
14101	CCGTCGCTTG	TGTCGCCAAG	TTCTGATGTT	TCATCAGCTG	AAAGCGCTGG	CAGGGGAAAA
14161	GGTTGCAGAA	GAAACACCGG	CGCTGGTTTC	CCGTCTTATT	CTGGATTATG	ACCTGAACAA
14221	CAAGGTTTCC	TTGCTGCAAA	CGGCCCGCAG	ACTGGCCCAT	GAAACGGACG	GTACGCCAGT
14281	GATGATGTCC	CCGCTGGAAA	TGGATTATCA	ACGTGTTAAT	CATGGCGTGA	CATGGAACG
14341	GCAGTCCATG	CCGCAGTTAG	AAAAAATGAA	CACGTTGCAG	CCATACCAAT	TGTTTGATTT
14401	ATATGGAGAA	GGAATTTCCG	GCGTTACTTT	ATCAGGATAC	TCAGAAAGCC	TGGTGGTACC
14461	GTGCTCCGGT	ACGGGATATC	ACTGCCGAAG	GAACGAATGC	GGTTACCTAT	GAGGAGGCGA
14521	AACCACTGCG	ACATATTCGG	GCACAACAGG	AAAGCGCGAT	GTTGTTGGAG	ATCAATGGTG
14581	ACGGGCGTCT	GGATTGGGTG	ATTACGGCAT	CAGGGTTACG	GGGTCACAC	ACCATGTCAC
14641	CGGAAGGTGA	ATGGACACCC	TTTATTCCAT	TATCCGCTGT	GCCAATGGAA	TATTTCCATC
14701	CGCAGGCAAA	ACTGGCTGAT	ATTGATGGGG	CTGGGCTGCC	TGACTTAGCG	CTTATCGGGC
14761	CARATAGTGT	ACGTGTCTGG	TCAAATAATC	CGGCAGGATG	GGATCGCGCT	CAGGATGTTA
14821	TTCAATTGTC	AAATAAGCCA	CTGCCGGTTC	CCGCCAAAAA	TAAGCTGATG	CTTGTGCGAT
14881	TCAGTGATAT	GACAGGCTCC	GGGCAATCAC	ATCTGGTGGA	AGTTACGGCA	AATAGCGTGC
14941	GCTACTGGCC	GAACCTGGGG	CATGGAAAAAT	TTGGTGAGCC	TCTGATGATA	ACAGGCTTCC
15001	AAATTACGGG	GAAACGTTTA	ACCCCCACAG	ACTGTATATG	GTAGACCTAA	ATGGCTCAGG

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Fig.2.

15061	CACCACCCGA	TTTTATTTAT	GCCCCGAATA	CTTACCTTGA	ACTCTATGCC	AATGAAAGCG
15121	GCAATCATTG	TGCTGAACCT	CAGCGTATTG	ATCTGCCGGA	TGGGGTACGT	TTTGATGATA
15181	CTTGTCGGTT	ACAAATAGCG	GATACACAAG	GATTAGGGAC	TGCCAGCATT	ATTTTGACGA
15241	TCCCCCATAT	GAAGGTGCAG	CACTGGCGAT	TGGATATGAC	CATATTCAAG	CCTTGGCTGC
15301	TGAATGCCGT	CAATAACAAT	ATGGGAACAG	AAACCACGCT	GTATTATCGC	AGCTCTGCCC
15361	AGTTCTGGCT	GGATGAGAAA	TTACAGGCTT	CTGAATCCGG	GATGACGGTG	GTCAGCTACT
15421	TACCGTTCCC	GGTGCATGTG	TTGTGGCGCA	CGGAAGTGCT	GGATGAAATT	TCCGGTAACC
15481	GATTGACCAG	CCATTATCAT	TACTCACATG	GTGCCTGGGA	TGGTCTGGAA	CGGGAGTTTC
15541	GTGGTTTTGG	GCGGGTGACG	CAAATCGATA	TTGATTACAG	GGCGAGTGCG	ACACAGGGGA
15601	CACATGCTGA	ACCACCGGCA	CCTTCGCGCA	CGGTAAATTG	GTACGGCACT	GGCGTACGGG
15661	AAGTCGATAT	TCTTCTGCCC	ACGGAATATT	GGCAGGGGGA	TCAACAGGCA	TTTCCCCATT
15721	TTACCCACAG	CTTTACCCGT	TATGACGAAA	AATCCGGTGG	TGATATGACG	GTCAACGGCA
15781	GCGAACAGGA	AGAATACTGG	TTACATCGAG	CCTTAAAGAG	ACAACGTTTA	CGCAGTGAGC
15841	TGTATGGGGA	TGATGATTCT	ATACTGGCCG	GTACGCCTTA	TTCAGTGGAT	GAATCCCGCA
15901	CCCAAGTACG	TTGTGTACCG	GTGATGGTAT	CGGACGTGCC	TGCGGTACTG	GTTCGCGTGG
15961	CCGAATCCCC	CCAATACCGA	TATGAAGGGG	TTGTTACCGA	TTCCACAGTG	CAGCCAAAAG
16021	ATTGTCCTTA	AATATGATGC	GTTAGGATTT	CCGCAGGACA	ATCTTGAGTG	TGCTTATTCG
16081	AGACGTCCAC	AGCCTGAGTT	CTCGCCTTAT	CCGGATACCC	TGCCCCGAAAC	ACTTTTCACC
16141	AGCAGTTTCG	ACGAACAGCA	GATGTTCTCT	CGTCTGACAC	GCCAGCGTTT	TTCTTATCAC
16201	CATCTGAATC	ATGATGATAA	TACGTGGATC	ACAGGGCTTA	TGGATACCTC	ACGCAGTGAC
16261	GCACGTATTT	ATCAAGCCGA	TAAAGTGCCG	GACGCTGGAT	TTTCCCTTGA	ATGTTTTCCT
16321	GCCACAGGTG	CAGGAGCATT	GTTGTTGCCT	GATGCCCGAG	CCGATTATCT	GGGACATCAG
16381	CGTGTAGCAT	ATACCGGTCC	AGAAGAGCAA	CCCCGTATTG	CTCCGCTGGT	GGCATACATT
16441	GAAACCCGAG	AGTTTGATGA	ACGATCGTTG	GCGGCTTTTG	AGGAGGTGAT	GGATGAGCAG
16501	GAGCTGACAA	AACAGCTGAA	TGATGCGGCG	TGGAATACCG	CAAAAGTGCC	GTTCAGTGAA
16561	AAGACAGATT	TCCATGTCTG	GGTGGGACAA	AAGGAATTTA	CAGAATATGC	CGGTGCAGAC
16621	GGATTCTATC	GGCCATTGGT	GCAACGGGAA	ACCAAGCTTA	CAGGTCAAAC	GACAGTGACG
16681	TGGGATAGCC	ATTACTGTGT	TATCACCACA	ACAGAGGATG	CGGCTGGCCT	GCGTATGCAA
16741	GCGCATTACG	ATTATCGATT	TATGGTTGCG	GATAACACCA	CAGATATCAA	GCTTATGGA
16801	CACACCGTGA	CGTTTGATGC	ACTGGGGACG	GTAAACAGCT	TCCGTTTCTG	GGGGACTGAA
16861	AACGGTGAAA	AACAAGGATA	TACCCCTGCG	GAAAAAGAAA	CTGTCCCTTT	TATTGTCCCC
16921	ACAACGGTGG	ATGATGCTCT	GGCATTGAAA	CCCGSCATAC	CTGTTGCAGG	GCTGATGGTT
16981	TATGCCCTCT	TGAGCTGGAT	GGTTCAGGCC	AGCTTTTCTA	ATGATGGGGA	GCTTTATGGA
17041	GAGCTGAAAC	CGGCTGGGAT	CATCACTGAA	GATGGTTATC	TCCTGTGCGT	TGCTTTTCGC
17101	CGCTGGCATC	AAAATAACCC	TGCCGCTGCC	ATGCCAAAGC	AAGTCAATTC	ACAGAACCCT
17161	CCCCATGTAC	TGAGTGTGAT	CACCGACCGC	TATGATGCGG	ATCCGGAACA	ACAATTACGT
17221	CAAAACGTTA	CGTTTAGTGA	TGGTTTTGGG	CGAAACCTTA	CAAAACAGCG	TACGCCATGA
17281	AAGTGGTGAA	GCCTGGGTAC	CTGATGAGTA	TGGAGCCAAAT	GTGGCTGAAA	ATCAAGGCGC
17341	CCCTGAAACG	GGCGATTACA	AATTTCCCGT	TGGGCAATTT	CCCGGACGTA	CAGAATATTA
17401	ACGGGAAAAG	GCAAGCCCCC	TGCGTTACGT	TTCAAACCGT	ATTCCTGAAA	TAATTTGGGC
17461	AACTATGTCA	AGTTGACCAA	AAAATGCCCG	GCAGGATATG	TATGCCGATA	CCCATACTA
17521	TGATCCGTTG	GGGCGTGAAT	ATCAGGTTAT	CACGCCAAAG	GCGGGTTGCG	TCGATCCTTA
17581	TTCACTCCCT	GGTTTGTGGT	GAATGAAGTT	GAAAAAGACA	CTCCCGGTGA	ATGACAGCAT
17641	AAAGCTCAGT	GATGCCTGTT	CACTGAACAG	ACATCACTCC	ATTTAGGAAT	GAATCATGAA
17701	GAATTTCTGT	CACAGCAATA	CGCCATCCGT	CACCGTACTG	GACAACCGTG	GTCAACAGT
17761	ACGCGAAAATA	GCCTGGTATC	GGCACCCCGA	TACACCTCAG	GTAACCGATG	AACGCATCAC
17821	CGGTTATCAA	TATGATGCTC	AAGGATCTCT	GACTCAGAGT	ATTGATCCGC	GATTTTATGA
17881	ACGCCAGCAG	ACAGCGAGTG	ACAAGAACGC	CATTACACCC	AATCTTATTC	TCTTGTCTAT
17941	ACTCAGTAAG	AAGGCATTGC	GTACGCAAG	TGTGGATGCC	GGAACCCGTG	TGCGCCTGCA
18001	TGATGTTGCC	GGGCGTCCCG	TTTTAGCTGT	CAGCGCCAAT	GGCGTTAGCC	GAACGTTTCA
18061	GTATGAAAGT	GATAACCTTC	CGGGACGATT	GCTAACGATT	ACCGAGCAGG	TAAAAGGAGA
18121	GAACGCCTGT	ATCAGCGAGC	GATTGATTTG	GTCAGGAAAT	ACGCCGGCAG	AAAAAGGCAA
18181	TAATTTGGCC	GGCCAGTGCG	TGGTCCATTA	TGATCCCAAC	GGAATGAATC	AAACCAACAG
18241	CATATTGTTA	ACCAGCATAAC	CCTTGTCCAT	CACACAGCAA	TTAGTGAAAG	ATGACAGCGA
18301	ACCCGATTGG	CACGGTATGG	ATGAATTTGG	CTGGAAAAAC	GCGCTGGCGC	CGGAAAGCTT
18361	CACCTCTGTC	AGCACAAACG	ATGCTACCGG	CACGGTATTA	ACGAGTACAG	ATGCTGCCCG
18421	AAAACAAGCAA	CGTATCGCCT	ATGATGTGGC	CGGTCTGCTT	CAAGGCAGTT	GGTTGGCGCT
18481	GAAAGGGGAAA	CAAGAAACAG	TTATCGTGAA	ATCCCTGACC	TATTCCGCTG	CCAGCCAGAA
18541	GCTACGGGAG	GAACATGGTA	ACGGGATAGT	GACTACATAT	ACCTATGAAC	CCGAGACGCA
18601	ACGAGTTATT	GGCATAAAAA	CAGAACGTCC	TTCCGGTCAT	GCCGCTGGGG	AGAAAAATTT
18661	ACAAAACCTG	CGTTATGAAT	ATGATCCTGT	CGGAAATGTG	CTGAAATCAA	CTAATGATGC
18721	TGAAATTACC	CGCTTTTGGC	GCAACCAGAA	AATTGTACCG	GAAAATACTT	ACACCTATGA
18781	CAGCCTGTAC	CAGCTGGTTT	CCGTCACTGG	GCGTGAAATG	GCGAATATTG	GCCGACAAAA
18841	AAAACCACTTA	CCCATCCCCG	CTCTGATTGA	TAACAATACT	TATACGAATT	ACTCTGCGAC

Fig.2.

18901	TTACGACTAT	GATCGTG GGG	GAATCTGACC	AGAATCGCAT	AATTCACGAT	CACCGGTAAT
18961	AACTATACAA	CGAACATGAC	CGTTTCAGAT	CACAGCAACC	GGGCTGTACT	GGAAAGAGCTG
19021	GCGCAAGATC	CCACTCAGGT	GGATATGTTG	TTACCCCCG	GCGGGCATCA	GACCCGGCTT
19081	GTTCCCGGTC	AGGATCTTTT	CTGGACACCC	CGTGACGAAT	TGCAACAAGT	GATATTGGTC
19141	AATAGGGAAA	ATACGACGCC	TGATCAGGAA	TTCTACCGTT	ATGATGCAGA	CAGTCAGCGT
19201	GTCATTAAGA	CTCATATTCA	GAAGACAGGT	AACAGTGAGC	AAATACAGCG	AACATTATAT
19261	TTGCCAGAGC	TGGAATGGCG	CACGACATAT	AGCGGCAATA	CATTAAAAGA	GTTTTTGCAG
19321	GTCATCACTG	TCGGTGAAGC	GGGTCAGGCA	CAAGTGCGGG	TGCTGCATTG	GGAAACAGGC
19381	AAACCGGCGG	ATATCAGCAA	TGATCAGCTG	CGCTACAGTT	ATGGCAACCT	GATTGCGAGT
19441	AGCGGGCTGG	AATTTGGGACA	GTGACGGGCA	GATCATTAGT	CAGGAAGAAT	ATTACCCCTA
19501	TGGGGGAACC	GCCGTGTGGG	CACCCGAAAT	CAGTCAGAAG	CTGATTACAC	AAGCCGCGT
19561	TATTCTGGCA	AAGAGCGGGA	TGCAACAGGG	TTGTATTACT	ACGGCTATCG	TTATTATCAA
19621	TCGTGGACAG	GGCGATGGTT	GAGTGTAGAT	CCTGCCGGTG	AGGCCGATGG	TCTCAATTTG
19681	TTCCGAATGT	GCAGGAATAA	CCCCATCGTT	TTTTCTGATT	CTGATGGTCG	TTTCCCCGGT
19741	CAGGGTGTCC	TTGCCCTGGAT	AGGGAATAAA	GCGTATCGAA	AGGCAGTCAA	CATCAGACA
19801	GAACACCTGC	TTGAACAAGG	CGCTTCCTTT	GATACGTTCT	TGAAATTAAT	CCGAGGATTG
19861	CGAACGTTTG	TTTTGGGTGT	GGGGGTACAA	GTCTGGGGGT	GAAGCGGCCA	CGATTGCAGG
19921	AGCGTCGCCT	TGGGGGATCG	TCGGGGCTGC	CATTGGTGGT	TTTGTCTCCG	GGGCGGTGAT
19981	GGGGTTTTTC	GCGAACAAAC	TCTCAGAAAA	AATTGGGGAA	GTTTTTAAGT	ATCTGACCGC
20041	TAAACGTTCT	GCTCCTGTTC	AGGTAGGCGC	TTTTGTTGTC	ACATCGCTTG	TGACGTCTGC
20101	ACTATTTAAC	AGCTCTTCGA	CAGGTACCGC	CATTTCCGCA	GCAACAGCGG	TCACCGTTGG
20161	AGGATTAATG	GCTTTAGCCG	GAGAACATAA	CACGGGCATG	GCTATCAGTA	TTGCCACACC
20221	CGCCGGACAA	AGTACGCTGG	ATACGCTCAG	GCCCCGTAAT	GTCAGCGCGC	CAGAGCGGTT
20281	AGGGCACTAT	CAGGCGCAAT	TATTGGCGGC	ATATTACTTG	CGCCGCATCA	GGGAAGTTCT
20341	GAGCTGGGTG	AACGGGCAGC	GATTGGTGCT	ATGTATGGTG	CTCGATGGGG	AAGGATCAAT
20401	GGTAATCTAT	GGGATGGCCC	TTATCGGTTT	ATCGGCAGGT	TACTGCTCAG	AAGAGGCATT
20461	AGCTCTGCCA	TTTCCCACGC	TGTCAGTTCC	AGGAGCTGGT	TTGGCCGAAT	GATAGGAGAA
20521	AGTGTGCGGA	GAAATATTTT	TGAAGTATTA	TTACCTTATA	GCCGTACACC	CGGTGAATGG
20581	GTTGGTGCAG	CCATTGGCGG	GACAGCCGCG	GCCGCTCATC	ATGCCGTTGG	AGGGGAAGTT
20641	GCCAATGCCG	CTAGCCGGGT	TACCTGGAGC	GGCTTTAAGC	GGGCTTTTAA	TAACCTCTTC
20701	TTTAAACGCT	CTGCACGTCA	TAATGAATCC	GAAGCATAAC	AATCATGTTT	ATTCCCACCT
20761	TGTCAATGGT	GACAAGGTGG	GTTTTTCGGA	TGTGTGGACA	GAGACCCGTA	CAGGGTCTCT
20821	GTCCAGTTAA	TTTTTGGATC	AAGAAGCAAT	GGTGTAAACG	ATATGCAAAA	TGATATCGCT
20881	CAGGCTGAGC	AATAAGCTTT	TCTGTTTACC	ACTGATACCG	GGAAACTGA	GGGTTAATGT
20941	GCCTGTATCG	GCCACAGGAA	GCCCTTCAAA	TGGCAGGTAC	TTAGCATCAT	TGAAATCCAT
21001	CTGGAATTGA	CCACTGTCT	TCATGCCATG	TGAGATCACA	ATCGCTTTGC	AGCCACGTGG
21061	CATCATTGTA	CTGCCGCCAT	AACTCAGTAT	TGCCCGGACA	TCCTGATAAG	GCCTTAAAG
21121	GGCAGGTAAC	GTCACTATGA	TTTGTTTGAT	ACGGCGTGTA	TTACCTTAAAC	CGTCAGGATA
21181	ATCGGTAGCA	ATATTTCAGT	CCGATAATTT	GAGGCTGGCT	TGCAGTTGTG	TCCCTTCGAC
21241	GTTCAAACCG	TTAAGCGTTG	TGCCTGCACT	GCCTTCACCT	GCATTGACTA	ACTCAGTCAC
21301	TTTATCTTTT	AAAATGAAAC	TATTTTCTGT	CAGACCAGCA	TACACTTCAG	CCAGAGAAAC
21361	GGTCTGTGTG	ACCTCCAGTG	CCCCTTCATC	TTTTTCCAAA	TAGCTTTTTT	CCATCTGTGC
21421	TAAATTCAGC	ATCAGGGTTT	CACCCGCTAA	TAAACCCGCA	TAAGTCCCAT	GCCAAAGCAC
21481	TGGTTTAAAT	AAGTGTGCTG	CCGATTATT	CAATTTCATC	TGATAAGTTT	GCTCTGCCAT
21541	TAAACAGAGT	GAGACCGCCA	AATCATAAAA	CTGATAATAA	ATAGCGGACA	ACGTTCCACG
21601	GAGCCAGTTG	TATAGCGCTG	CATTACTGAA	TTTACTTTGC	AGAAAGGCTA	ACTGCGCCTG
21661	AGTTTGTGCC	TGCTGAGTTT	CCAGATAGTT	TTTTTGTAAAT	ACTGCCGCTT	CACGACGTAC
21721	AGCCAGCGTC	GCTAATTGAG	CATCAATTTG	TTTTATCTCA	GCTTCCGCAT	TATTGCGCTG
21781	AAATTTCCAC	TCTTGCCGAC	GGCGACGGTA	TATTTCTGAT	TGGCTGATTT	TGCTCGCGGC
21841	AAATACGTGT	GCTGACGCAG	AAATTTTCAT	ACCAATCGCA	CTGGCATTGA	AAAGCGCCCC
21901	AAAACGGGAA	CCTCCACAG	CAAAACCGTA	AATATTGGGG	ACGAGATCTG	CCGCGCGCGC
21961	GGCCATATGC	AGGGCTGTGC	CGCTGGTGCT	CAAGACCGAT	GAAGAGAGGT	AAAGATCCAT
22021	CGCTTGTTTT	TCACCAGCGT	TAACATCTTC	GTGATACAGC	GTATTGAAAC	TGTCAAAACG
22081	AGACTGTGCA	CCATGACGGC	TTTCTTGAAG	CGCCAATTTA	TCAGCATCAA	TTTCAGCCAT
22141	GACCTTATCC	TGCATTTTAA	TACTTTGCAG	GGCTAACTCA	CTGCCCTGAG	TTTGCAGTAT
22201	TTCAGCCAAG	GCTTCTGCAT	CCTGCCGTTT	AGTAATGCTG	AGCAGGGTAT	TGCCAAATTG
22261	TATCAACTGG	CTTACCCCCC	ACTTGGCATT	TTCCAGAAATC	ACCGGAAAAC	GGTACATCGG
22321	CATCACTGCA	TGAGGTAAT	CGCCGCGGCC	TTGTGAAGCA	GTGATGGCAG	CACTGAGTAA
22381	CATGGACGGA	TCTGCGGGCG	TGGCATAGAG	AGATAATGAC	AGTGGCTGAC	CGTCCGATTG
22441	CAGGTTATGG	CGTAAGTTAT	AGAGGCGTTG	CGTCAATGTC	TGCCAGTAAC	CTTGCAAGTTT
22501	TTTATTAATT	TGAGGGAGGA	ACAATGCGGT	TAACGAAAT	TGCGGTACGT	TTCTGGGGTA
22561	ATGCAGCGCG	CTGACGCAGT	TGCAGCATTT	TATGTTGATA	ATGATGCCGC	ATTGTTTGCC
22621	TGGCAGCTTC	TTCCAGCCGT	GGCTCTGACC	AATCGTTATC	CAATGAAAAA	TAAGGCTCAT
22681	CACCCAAATA	AGTGAGCGCC	TGTACATACC	ACATTTTAGC	TTCTGTTTAA	GTATCAGGTT

Fig.2.

22741	CAAGCTGGCG	ATAGGCGCTA	TCTCCGCGGG	TAATCAACAA	ATCCAGCATT	TTCATAAAGG
22801	TAGCCACTTT	ATAGTGCATC	GGATCATGCT	GGGCAACGGC	GTCCGGATCG	ACCGAATCCA
22861	GCGGATTGGC	ATTCCAGGAC	GTATCTTCCT	CCAATGGGCG	GACGTTCCAG	TAATAATCCT
22921	GCATTTCAAC	CTGAACCGAA	TATCCGGTCG	GGTTCAGATA	TAGCGCAGCC	AGCGTGTGGA
22981	TCCGGTAAAA	TCTGCTCTTG	CAATAAGCGC	TGGAATACCA	TCATGGGCGT	TGTAATAGAA
23041	CAATCCCAAG	AAATAGATTG	CATTGGCGCC	GTTTGAAATC	CATGGGTTCA	GTGTTATTTT
23101	TCATGACACG	ACTTGAATAC	CCCTTTTATA	TTTTTTGATA	TTTTTTTACTA	TCCCTGTGTG
23161	TGTCATTCCC	GAATCATGAT	CGGCATCATT	AGTGAATATA	AATTGATTTT	TCGTCTCATC
23221	AAAATAAAAG	AAAGCAGATT	CCCAGGATTT	GTCATAGATA	ATTTTTTGTG	ACCCAACCCC
23281	TAATCTGACA	CCTTCACGTA	TGTAATATCC	TTTAGCATAG	GGAACAAAGA	GCGTTACTGT
23341	GGTTTCAATA	TCAGATAACA	TTCTTTCGTA	ATAAGGTTGT	CTGGCAGAAAT	TGCCATCAAT
23401	ATTCCCAATA	TGGATCTTAA	ACCAACGTTT	ATCACCATGC	TCCTCTTTAT	TGTAGGGGGG
23461	CAACTTAAAT	GTCCGATAAA	ACCCTTCACC	TAATTGCGGC	TCTGGTAAAT	TTTGGCTTTC
23521	CATACTTAAA	ACATTATCAA	TACCAATATT	GGCTCTTTCA	GCTAATTTTC	TGGAAAATAA
23581	AGTATTTAAC	CGGGTTCGTG	AAGGGCCAAT	CTGCATATAT	TGTGTGCCCTG	ATGGCATTIT
23641	ATGCAGTGAT	ATAACGTTAC	TTGTATCTTT	GGATTTTAGT	TTTATATGAA	TTGGCGATTTC
23701	AATAACAATA	TCGTTATAAC	CGCCGTCCGG	TTGCTTAATA	ATAAACTTCG	TCCACAGAGG
23761	AATATCATAG	CCTTCAATAT	CAACTTTTAC	TTGATTAAAA	TCATATACCA	TAGGGTCAGA
23821	TTCGTGTGAA	GGTTTAGATG	CCACATGGTC	TTTCAGCATT	AACTCCACTA	GAATATCAGA
23881	GCCATTTTTT	AATAAAAAAC	TAATGTTTTT	ATCTTGGATC	TGTTTCGATC	TAGATGAAGC
23941	AAGTTTTTAT	ATCTGTGGCT	GGTTGAACAT	AAATACACCC	ATGGATCTCT	CCGAAGGAAC
24001	AGTGCCGCAA	TATTTCCCAT	GTTATTAATG	ATTGAAACAT	CATTAGTAAA	TGATTCACAT
24061	ATATATATGC	ATACTCCTGT	GTTATCTTTC	CAATCTAATA	CTATGTTAGT	ATCAAGTTTG
24121	AATTCAGCAT	CATCTGATTC	ATAATCATAA	TTTATACCAA	CTCCAATTTC	TGATTTTCTA
24181	GGAATTTTTT	CCTTGGTTCT	TAGATGCATT	AACACTCTAA	AATATTCGGC	ATTTTAAAGA
24241	TCGATGGAAA	TAATAAAATC	CAAAGTTCCA	TAATGAAAAA	CTTCTTCTTC	TTTTCCAAGC
24301	ATTTTCATCAT	GTCTATCATA	ATCAAATAAA	ATAACCGTTT	CATCTTCTAC	CATCGATAAC
24361	AGGTATTTAA	CCTCATCATT	ATATATATTG	CCTTTTGAAA	AATTAATTTT	CATTGAAGGA
24421	TTGAACGTTA	AATTAATATG	ACCATTTCCT	GGTGATATAT	ACGAGAGTTC	AAAAATATTT
24481	CCGGTAAAC	TGGCTAATTT	ATTTTTGTG	GTTATAGATT	CCTTATATTC	GGCCAAATAA
24541	TCTGTAGCAA	ATTGATTGTT	GACTTTGTAT	TCTGTCTGG	TATCAAGTTC	TGATAATGTG
24601	CTCTTAACAA	TGGCGTCTAA	ATCATTTCCT	GTGAGAATGG	ATAATGTCTA	ATCAGGGTTA
24661	ATGCTCATCC	CTTCTCTTGC	AGGAAGACTA	TTAAAAGAAAT	AATTGTCTTT	TTCTCTATGG
24721	AAATAAACAA	TAATGACGTC	TTTTTCATAA	TCAGAAGAAC	AATACATACC	AATGCTGGCT
24781	TTTTTATTGA	TCAGGTTTTT	TATTTTATCA	GTCACATTAA	AATTAACCGG	TGAGCTCCAG
24841	CTGCCATCAT	AACGAATATG	TGACAGTTTT	AATATATAAT	CAGTGATATC	TATCTTGCCA
24901	TCTTCACITT	CATTTTTCAG	CTCTTTTGT	TCCAGCCACA	GTAAATACAA	ACGAGACTTG
24961	TAAATAACAG	GTCTGATATT	TTCTGCCAT	ACATTGATGG	GTATTTCAAT	TTTTTTCCAT
25021	TCTCCCCAGG	CATTGGCAGC	AAATTGACCG	TGCTGGCACT	TTTGGTGATC	GACATTGCGC
25081	CAATAATATA	TTCTGGGTTT	TGCTGGGCTA	TAACCAATTA	AATAAGTGAG	CCCCCTATTG
25141	ACATTAATAC	TGTCATGATA	TCCGCTAATC	ACCTGCAAGT	TAGCGACATC	TTCAAATGCG
25201	GTCAGATAAT	TTTTAAAGCT	ATCTTCAACG	GTATCGATAT	TTAACTGACT	TTGGGAAAGT
25261	TGCTGTAACA	GGTTGTTCAT	CATACCTGTC	TGACCAATAC	GAATCGTGGG	GTGATATAG
25321	TTTCCGGAT	AATAGGCCAG	TTTCAGATACG	CCGGCCAGG	TGCTATACCG	TCGATTGTAG
25381	GTTTCCAGT	CGCAGAAGAA	CTGACGGGTT	TTCACTGGCT	TTGATACTTT	TCTTCAACA
25441	TTATTCAACG	CCCGTTTGAC	ATATACTGA	ATGCTGGCAA	TGGCTTCTGC	CACACGGGTG
25501	GTTTTCACTT	GGGCAGAAAC	TTGGTTATCA	ATCAGCAGAT	AGCTGTACAA	CTCATCCCGG
25561	CTCTTAATCT	GTTGAGGTGC	ACCATTTTTG	ATGTAGTAA	CCTGGCCGC	TGTCGTCTGT
25621	GCTTCATCCA	GCCATGCCTG	AAGCTGGTCG	GATTGTTGAC	TGTTCAATCC	CGCCTGCAAC
25681	AAAGTACTGG	CGGCTTGCCA	ATCATCAAAAT	GTTGGCATCG	GGGTTTCCGG	TTCAACGACA
25741	TATTTTAAAT	TTATGAGTGC	AGCAACACCA	TCCGGGGTAA	TACCCAATGT	AGCAGCGACA
25801	TCCAGCCATT	GCAGAGTGAC	ATCTATAAGT	TCTCCAGTTG	GTAAAGGTAT	TCACTCCCAA
25861	ACCGGTCTGT	TGCAATGCTT	GTGTCACAAC	CTGAGCATCA	AAATTTTAA	GCCACCGCCA
25921	AATTGTTCCG	CAGTCAACGC	TCCTAAGTTC	CAAATGCTGT	TAAGATTCTG	TCGCGTAGCT
25981	TCACAACGCA	TGATCACAGC	ATGGAAGCGG	GTCAGCGCTT	GCAAAGTGGG	GAGATCATGT
26041	TGCAGTGCTG	TGGTTTCTGA	TTGGAATTTT	TCCGGTTTTG	TCACCAACAG	GGTCAGTTTC
26101	TTTTCGCTGA	GTCCAATATT	GCGCACAATC	AGAGAAAGTT	GCCCCAGTAC	CTGACAAAAA
26161	GCCACCATGT	TGCTGGTTTC	ATTCTCTGAG	CGATCACGGT	TAGCCGCAAT	AATCATGAAA
26221	TCATCGAATG	TCAGTCTTGT	TGGTTTTATC	TGATTAATCC	ACAGCAAAAT	AGTTTCTGCT
26281	GTTTGGCTG	AATCCATTTG	AATGCTGGCA	GCAATCAGCG	GGGCAGCTGC	ACGGATCAGT
26341	TCGTCATCAC	CGAGTGAAAG	TGTTGATAAT	CCATTACTTA	GTGTCGTGAT	AAGGTTTTCA
26401	ATATCCGGCG	TAAGGACAGT	GCTGTAATTA	TCCGTGGTCA	TCAGAAACAC	ATCACTGACA
26461	GACCATTTCT	GTGTTGTCAG	CCACTGGGTG	CATTGGAACA	GAAAGCTGAT	TAATTGCGTT
26521	AATGCTGTAT	CAGAAAAAAG	GGCAATTTTC	GTGTTACAT	AGGGAGAAAC	CGACAACAAC

Fig.2.

26581	ATGGATAATT	CATTCACTGT	CAGATGATGA	ATGTCTGCCA	GCAGACGAAC	GCGATAAAGC
26641	AGAGACAGGT	TCTCGATGGA	ACACATAAAT	TCTGGATTGG	TTCCGCCATT	AGCCAGTTTC
26701	CATAATGTAT	ACAGTTCACT	ATCAITCACT	CTGAAAGCAC	GTITTCATTAT	TCCCAAATAA
26761	AAATGGTTTT	TTGATTCCACC	GGGGGTAAA	TCCAGTTTGG	TATTATCAGC	AGAAAACCTCT
26821	TGGCCATTTA	ATAGCGGTGT	ATTGAACAGC	ATTGTAAAAT	GACTGGGTTC	TTGTTTAGTG
26881	GAATATTGGC	TGATATCTGA	ATGACACAAT	ACCAGCGCAT	CGCTGACGCT	AATATTATAG
26941	TGCTGCATAT	AATATTGAAC	ATAAAACAGC	TTACCCAACA	CATTGCTGTC	AATGGTTAAG
27001	TCATCATAAA	TACTTTCTAT	TACTTGCCAG	ATATCTTCTG	GAGATATGCC	TGTGGCTTTA
27061	TACAAACGAA	TCGCTTTTAT	CAGCTTTAAC	AGGAATATAT	CACCGGGAAC	TCCATCATTT
27121	TAAAGTGTGC	ATTGGCATTG	ATAGCATCCG	ACGGATTGGG	TTAACTCGCC	ATAAGCGGAG
27181	TGTTATACCG	TTGGTGATTT	GCTCTGTCGT	CAATTTAATG	GGAACTACTGT	AATGGGTATT
27241	AGCAATGGGG	ACGAAATTTT	TATCTTGGTA	TATATATTCT	TTATCTCCAT	TCTGGAGACG
27301	AAAATCCAAG	TGGTCAGGTT	CTGTTTTTTT	TACACTGAAA	TTATATTTGT	ATTCAATTTT
27361	TTTGATTGGA	ATTAGCTCTG	CATAGTTTAA	ATGTGAATCG	TAGAAATCTT	TGCGGGTTCC
27421	CTTAATCAAT	CTTGCCGTTG	CCGTATCATT	CCCGTCATTG	ACCAATGTTA	TCAGTTGCTC
27481	ATTCTTATAC	TGTTGATTTG	TATTTTCTT	ACCGAAGGAG	AGATTGACAA	ATAAAGCTAG
27541	TTCATCATAA	GACAAATCGT	AGTAGCGAGC	CAAAGAAGCA	TAACTCTTAA	AAATCAGTAC
27601	ATCATCTGTA	CCGAAATTTT	TCTTCATCAG	TTCTGTGAA	TTTTCCGGTG	TAATTTCTTC
27661	TACAAGGATT	TGATACAATT	CAGGCGATAT	ATCAGTCTTA	ATAGCCAGTA	GCGATGTTGG
27721	GTCCATTAAT	TCCGCTACGT	CTGTATTACG	GCTAAATGCG	GTGAGATTAT	TATCTTGCAA
27781	TAAAATTGCC	TGACGGGCTG	ACTCATACGG	CAGATGATAG	GGTGTCTATG	CGGTTTGCCG
27841	GTAAGTGGAC	AACATTTTCA	TTACACCGTT	ATAGTCAGTT	TTCTCTAACG	TCTGAATATT
27901	ATGCAGCAGT	AATTCATTAG	ATAAGGATAA	TGTGGAAATT	TCTTCATCCA	TATTATTCTG
27961	TGTCAGTGCC	AGTGAAGCAA	TGTCGGGGCG	TGCTTTATTTC	AGGTGATATT	GAGAAATTGT
28021	AGGATGAAAA	TCTTTGCTTT	CCCGATATAA	TTCTGTAAAT	TAAGCCGCTG	GTGAAAATAT
28081	GGAAGCAATT	GATCCCGGTT	TTACAAAACG	GTGGGCGCGG	CCATAAAACC	AACGTGTTGA
28141	ACTATTGTTT	AGGGTTGACG	GTGTAATATT	AAGGTTAGTG	ATATTAGCCA	GTTGTGGATT
28201	AGCACGGGAC	AAAATGCGCA	GTTCTTCAAG	TTTATTCTGT	TTTGATTCTT	GATGACCGTG
28261	TTGATATAAA	AAGTCTGTTT	CTCGCCACGT	CAGAGTTCCA	CTTGTCCTAT	GACGAAATTC
28321	GCTGAAAGAC	ATAAACGAAA	TGTTTGTCAA	TAATAAAGTA	TCACCAGCCT	TTTTCTATTT
28381	ATCTTATCTA	ACAGTTTCATT	AACTTTTATC	ATATAAATCC	TTAAGTTATT	GTCAATTTAA
28441	TGATTAATGG	TTTTTAGGTT	GAGATTATTA	TAATCTGATA	GGAATATTAT	GGTTAATTAA
28501	ATTGATACTG	ATTTATCGCT	CTATTCTTTC	AATAAAAAAT	AAAGAACCTC	CCTATAATAC
28561	ATGGATTAA	ATAATGAATA	CCGTATGTTA	AAAATTTAAAT	TTTAACAAAC	TTTCATGAAA
28621	AAATTCAACT	CAACAATTGT	TTAAATATTT	TTAATTGTGT	TTGTGCTGTT	TGAAAAATGA
28681	ATGACTAATA	TTTATCTATG	AAAGATTATT	TATTGAGGAT	GTCTTGCTTG	GTTTGAGGGG
28741	GCTACGTTGG	AGTCAGATAA	ATGTGTGCAA	AAAGAAATCC	TTAATAAAGT	TGCGTAATTA
28801	CAAAAGTTGG	TATATCGTGA	CAAGAGTGAT	AGTAATGTCA	CATAATTTAT	TGAATACCCG
28861	AACCTCGCAA	ATGCGGGGTT	TTTCTTCGCA	TAATCAAAGA	GAAAGCTATG	AAAAAACAC
28921	TGATTACTCT	TATTCTCAGT	ACCCTTCTT	TTGGTGCTTT	GGCACAGCAG	GGTGGCTTCG
28981	TTTCCCGGA	CAGCACAGAC	TATACTCAGG	GTGGATTTAA	AGGTCCAAC	CCCAACCTGA
29041	CCAGCGTTGC	TCAAGCAAAA	TCTTTTCGTG	ATGATGCGTG	GGTTGTTCTG	GAAGGAAACA
29101	TTGTTAAACA	GGTTGGTCAC	GAACCTATG	AATTCGCGGC	CGCATAATAC	GACTCACTAT
29161	AGGGATCGCT	TATTACGGAC	TTATCCGGAA	AGCTATCTGG	AACCCCTGTT	ACGCTGAAT
29221	AAAACAGAA	TCAGGGATAA	CAGTGGTTCT	GTTTATGTTG	ACATTGATGA	TAAGCGCTGG
29281	ATGGGTCTGA	CGGCCACTCC	AACTGACAAA	GTTCTGATCG	AAGGTGAAGT	GGACAAAGAC
29341	TGGAACAGTG	TTGAAATTGA	TGTCAAAACT	ATCCGCATAG	TGAAATAACT	CAAGCACTTT
29401	GAATATAGCC	CCGCACTCGC	GGGGTTTTTT	GCTTTCTGGG	AGTCGGAAGT	TTAACCGTAG
29461	TGACGAGGAT	CAAAACTAAG	TTAACGGCAG	TGGTCACTGA	TTTGGTGAT	AAGTTATCAA
29521	AAGTTAAAAA	TCAAAACTTA	TTTTTTATTT	AATAGAGGAA	TGTCACCCTG	TAGGTGAATA
29581	ACGTTGACGG	ATGTAAATAT	ACAGTATTAT	AGTCCTTTGA	TATGTTATTA	AATTGAAAAA
29641	CCTTTAAACT	ATATTGCGGG	GAAATTATTA	TGTCAGATGT	TCGTAATATT	ATTAAATGTT
29701	ATAACAATTT	TGTTTGTGAA	TATAAAGCGG	ATTTATTTAA	ATAAGTTTTC	ATAATTGTGA
29761	TACACCCATT	TTTCTCATCC	CCGGTTTTTG	CTGTTGTAAG	GAAGCGGTTT	CCATGAAGAT
29821	TTTGACATGG	TTAAGCAACT	GCCACATAAA	TTGGCAGCAG	TGGTTTCTGT	TCACGGTTTT
29881	ATGCAAGGAT	TGCCATAGAC	GTTCATTTTT	ATTCACCCAC	GGGCAATAGG	TCCGTAATAA
29941	GAGAAGATTA	AATTTGGGAT	TCTTTGCCAG	CCAAACCCCT	ACCTTCCGGC	TCTTATGAAT
30001	GCAATAGTTA	TCTAAAATTA	ACGTGATGGT	TTTGGCATT	ACATATTGAT	TGTTAATTTT
30061	ATCTAACAA	TTGATAAATA	AATCTGAGTT	CTTTCTCAAG	CTACCGACAT	AAGTGATTTT
30121	TTTCGTTTTT	CGGTTGAGGC	AATTGGCAAG	GTAGTGTTTT	TGGTTCTTTT	CGGGGGTAAC
30181	AACACGCTTT	TGTTGCCCTT	TGAAGCACCA	GTCTGCACCG	ATTTTCTGGT	TCAGGTTGAT
30241	GTCCACCTCA	TCCTCATAGA	AGACCGGGTG	TTTCTCTTGA	GGCATTGGAT	AACGTCTCCG
30301	TGATTTTTGC	CATTTTTTCA	TCATACTCAG	GGTCAGGCAA	TTTTACGGTT	GGTCCGCCCC
30361	TTCCGCAAA	GATGCCCGTC	CGGCAAAAGT	AGCGATAGAG	GGTACTTTGA	GAGAGCGATG

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Fig.2.

30421	TATTCAGTAG	CTCATTGATT	TTAAGTGTA	TAAGCTCAAG	GCTCCATCGT	GAACGGAGAT
30481	AGCCAAATG	TTGTGGCGAG	TGCTGTAATA	AGAAAGAAAT	GACTGTGAAG	AGCGGAGCTA
30541	AGTTCCAGAT	GGCAGGCCTT	CCCCCGGGA	GGCTTTTAAG	TCCTTCCAAC	CCGTATAATG
30601	TTAACCAATT	TACCCAACGA	TGAACGGAAG	AACGTGAACA	GTGAAGCGTT	CTGGAAACGT
30661	GAGAAACCGT	ACTCCCTTCA	TGTAACATCA	AGAGCGCGGT	GAAGCGACGT	GCATAGTCCT
30721	TATCCCGGGT	TTTCTGGATA	GCTTTTTTCA	TCCGACGTCG	TTCATTTCGG	GGTATTGATG
30781	TTATGATTGG	CATGACTCAG	TCCATTTTGG	GATTTGTTTT	GATTTGGCGA	TTAATCAGAT
30841	CGCGAAAATC	GGACTGAGTT	CCCTTCAAGT	GATCTACTAT	TTTGAAATCT	TATTAAATCA
30901	GGAGTCAGCA	AATGAGTTAT	TCCCCATAAT	ACCTGACCAT	GTGGTTGTTT	ATCCGGGAAA
30961	TGATTTCATCT	ACCGGTGGTA	TGTGGATTCC	TTGGTGGCAT	AGTCAGAAAG	ATATTGACTC
31021	TGGCCATTAT	ATCAAAGTTA	CTTTCAGTAA	AAAGGACGCT	GCTGATATTG	TGAACTACAT
31081	GTTTCAACAT	GGCAGTTATG	TTTATTTTAC	AGACAGTAGT	AAACAATTGA	GCAATAAGCA
31141	AATTATGTCT	GGTGATTCAG	CTAAAGGCAA	AGGGGATTAT	AAGCTTGAAA	TTAAACAAAA
31201	CGGGAACCTT	CCACTGATGG	TATTGAATAA	ATATTGATTTC	ATTATTATTT	ATGGATAAGA
31261	AATTAAAGTTT	ATATTTTCATC	TGGTTTCTGC	AATTAAGTTT	TAAAAATTAA	TTCTACTTTT
31321	TTTATGGTTT	TATATTTAAT	GCCAATCATA	TTATTTTCTT	TATAATAATT	GATAGTTTAT
31381	TTATATAGTA	AATAAAATCT	GTTGGATGTG	ATTATTATTG	TGAGACGGTA	ATAATTAACA
31441	TAACAGAAAA	TTCATGGTTA	GGAAATTCAA	TCAACTTTTG	TCCGGTTTCC	TGACCATGAA
31501	GAGCTGTATT	TACTGTAGAA	CTCGCATTGA	TACTGGATTG	ATTAGCCGGA	CGAGTGTGGG
31561	GTCAGCAGAT	AATATGTTGT	ATATTGGCTG	TGGATTTTTC	AGCGAGATGA	TAGCTTTGGC
31621	AGTAAAGGCG	ATTAATAACC	GATAAAACAG	AGAGACGGAT	TGTGGCCAGG	AAAGCAAAAA
31681	AGCCTCACCA	TGACGCGTTA	TTCAAACATT	TTTTAACCCA	ACCAGAAACC	GCCCCGGAAT
31741	TTTTATCCCT	TTATCTGCCG	GAAGCGATCC	GGTCAGTGTG	TGATTTACCA	CACTAAAAC
31801	GGAAACCGCA	GCTTTGTGGA	CAGGCAATTA	CGTCAGTTGC	ACAGTGATGT	GCTGTATTCT
31861	GTCGAGACAA	CCCACGGGGA	CGGTTACATT	TATTGCCTGA	TTGAACACCA	GTCCACGCCT
31921	GATCCGTTAA	TGGCCTGGCG	GCTGATGTAT	TATTGCTGTG	CAGCCATGGC	TGCGCATCTG
31981	AAAAAAGGAC	ATACTGAAC	CCCTTTGGTC	GTCCCCCTGC	TGTTTTATCA	TGGTGAGGTG
32041	AGGCCTTACC	CTTACTCAA	TGATGGCTG	GATTGTTTTA	CACCTCTGTA	ACACCGCGCT
32101	CACCTGTATA	ATCAGCCCC	GCCGTTGGTG	GATATCAGTG	CGCTCAGTGA	TGAAGAGATC
32161	CTGACACATA	AAAGCATTGC	CTTGATGGAG	CTGGTACAAA	AACATATCCG	TTGCCGGGAT
32221	ATGCTGGAGT	GGGTTCCCCA	ATTGGTGGCG	TTGTTGAATG	CCGGTTATAA	TAGCCGCCAG
32281	CAGCGCCATG	TTGTGTTAAG	CTATATTTTA	CTGAATGGAC	ATACGCTGGA	TCTCGCCGAG
32341	TTTGTCCATC	AACCTGACTG	ACAATCTCCG	GAGCATGAAA	CCATGTTGAT	GACTATTGCA
32401	GAACAGCTTG	AACAAAAAGG	GCGTGAGCAA	GGCCGAGCAG	AAGGCAGAAC	AGAAGGCAGA
32461	GCTGAAGGAC	GGGAAGAAGG	CAAGCTGGAA	ACGGCGCGCG	CATTATTACG	GCATGGTGTG
32521	AGTCTGGACA	TCATTGTCTC	CAGTACCGGC	CTGAGCCCGG	AGAAAAATTGA	AGCGTTAAAG
32581	CATTAAATGG	ATACGCTTTT	TCACAGCAGG	ATATGGTGAC	CCCTGTGAGG	CCACCGGAAA
32641	ATTTTATTTA	CTACGATTTA	CGACGGGTTA	CTTTAGGAAG	CTGAATGAGA	CGTCTTTTGT
32701	TATATAACGG	TCCCATATCA	ATCTTCTCTT	TTCCGCGTAC	AGGTAAAGTA	CCCAAACTTT
32761	CGTGAGCAGC	ATTTGCCAAC	AGGCCATCAT	CTGTATCGCC	TGACCAAGAG	AAGATCCCGC
32821	CCAATTTTAT	TTTGGTTGCA	TAAATCCCTT	TATGCAGCAC	AGTGCGGGGC	GATCCAGTGT
32881	AAATCCAGTG	ACCACCGTCA	GCATTAAAGA	GTGCGTCAGC	GTCGGTTTCC	GTGTCTGTCA
32941	CCAGTTCAAA	CTGATTTTTC	CCGCGTGCAA	TTTCATATTC	CGCATCGTAT	TGGTTATTCA
33001	GCAGACAGAA	GAATTCCGGA	GCACCTTTTT	CCATCGTGCC	CAGTGGCTCT	CCTGTCTCTG
33061	TATAGCGGCG	CGTTGTCAGA	TCAGCACCCA	GACATGAACG	TCCATAGTTA	GCAAAATCCGA
33121	GGTGAATTTT	CTCCGGTTGT	ACACCTTGTG	ACAGTAAAAA	GCGGATCGCC	TCATCTGCCG
33181	AGTAATCCAT	GTCCCGATCA	GGATTGGGCG	GAGGAGGGTT	ATCGCCGTCA	TATTTCATATC
33241	TGGGGGGGATA	CAGGTTAGTA	TGGTGACCGA	TGTATTCTGC	CCAACCGGTA	CCAAAGAAGT
33301	CGTAGGTTCAT	CACAAAGATA	TTGTCTAAAT	AAGGTGCGAT	TTCTTTGAAG	CTGGACTTCT
33361	CCATTTTGGC	AACGACGGCG	CTACAGGCTA	TCGTGATTTT	TTTACGGGCC	CGGGTTCCAA
33421	AGGCGATGTT	CAGTGCTTCA	CGCAGCTCTT	TCACTAACAA	AACATAGTTT	GGGCCATCAT
33481	GTTCCGGGTC	GAATTCATTA	CCTTCTTCAC	CTGTGGCGCC	GGGGTATTCC	CAGTCGATAT
33541	CCACCGCAGT	AAACATGGGA	AAACGCCGGG	AAGAAAGTCGA	CGATGCTACT	CACAAATGTA
33601	GCACGTTGCT	CAGGATCTTT	GGCCATCACA	GAGAAATACC	CTGACATACT	CCAGCCGCCG
33661	ATACTGAATG	CGAGTTCCAG	CTTATGCCCT	GCCTGTTTTG	CTCGCGCTTT	CAGATTACGC
33721	AATCCCCCCA	GTAACCCGGA	GGCTGCATCC	TGATTGTAAT	ATTGCAAGAA	ATTCTTCGGG
33781	CTGGCATCAC	GGCGCTGATC	CGCGTCCAGA	CCGACATTGC	GTGTGGTGCC	TAAATCACCA
33841	TAAGGATCAA	CGGGTACAAT	ATGGCCTAAT	GTAATAGGGG	CAATCTGGCC	ACTGCTGGCT
33901	TCTGCTTGCC	GGTTCCACCC	GTCAACAACC	TCATTAATCC	GTTCCGATAA	CTTGCTTTTG
33961	TCACCGTTGA	CGGCCATAAA	ACTGAAAATC	AGGCGGTGCT	AGGCGGTAGG	CGGGATTTTT
34021	TCCAGATCAA	AACCACGGCC	GGGGCATCG	TCGCTGGTCA	GCGCAGTGTT	ATCCTGGGTT
34081	TCTGGCGACA	AACGCGCATC	ATACTGGCAC	CAGTCAGTAA	TATAGGCAGA	GACTTTAGGC
34141	AGCGGTTCTG	TATTTTCCGG	ATCAACTTCA	TATTCGTTGT	ACAGGGACTT	GGCAACACGT
34201	GCTGAAGAAT	AACCTCAAAG	AGTTCCGCTG	CCGTCAGGTT	TATATCCAC	CTTCTGATAG

Fig.2.

34261	GTTTCTTCTG	TGAGTGCATC	ATATTGCAAT	ACCTCGGTTT	TTTCTCCCGG	CGGTACATCA
34321	GGCGTATTGG	GGTTACCGTG	ATCGGCAATT	TCTTCCGGTG	TCGCCTCACG	GACATATTGC
34381	CAGGCATTCT	CATAAACCGG	TAAATCAGGT	GAAATATTGC	GGTCGGGAAT	ATGCCAGCGT
34441	TCAACCCAGC	CGATGTTTTT	AAAAACCGCG	CTATCATAAA	TGACATACCA	GGTTTGACCA
34501	CCAGATTGAT	TCTGCCAGGC	AACCAGAGAT	GCGCCTACTT	CGCTGCTGGC	GTCAGACATC
34561	GCTTTAATTG	AAGGGTATCG	ATAAACATTT	TGAGACATAA	TTTCACTTCC	GGCCCCGTTA
34621	TATTCGGGGG	CCGGCTCCTG	ATATCAGTTA	GAATTGTCTT	GTTTTAATTG	ATGTTTTATT
34681	AGACGGCTAC	GAACCTGCTG	GCTGAATCTA	TTACTTCCGC	CACTCACATC	ACGCGCGGTA
34741	TAACGCAGAT	GGAGGATAAT	ATCGCTCAGC	GACTCCAGCA	GCTGATCCTG	ATCGGAACCG
34801	AATTCCAAC	TCCACTGTGA	AATGGCGCCT	GTCCTTCAA	AAGGCAGGAA	AAGTTTCATCA
34861	TCAAAATTGA	GCCTGAACAT	GCCGCTGTCT	TCCATGGCCG	TTGAAATCAC	CACACCTTGA
34921	TTAGCCTGTA	CGTTCAGCAA	AACGTTTTTC	GGTTTGGTGT	ATTCCAAGG	GTTAAGCAAA
34981	TAATCGATAG	TTTTTAAGTC	AGCAGTACTG	TAAAGCGTAT	TGCTGAGTTG	TACCAGTGAA
35041	GCCCCGTACAT	CTTCATAAGG	CCCAGCAAT	GCGGGCAATG	ACAGCGCTAC	GGTTTTTATA
35101	CGCCGATCAG	CGTGGGTCCG	ATAATCGCGC	AAGAACATTT	CGGCGCTCAG	TAAGAAAGTG
35161	AATGAACCCG	TACTCTTGCC	AATTTCCAC	TGTGATGATG	TCAGTAATGA	TTTTACCGAT
35221	ATGGTTTTTA	TGATCTCCAG	ACGTCTGGTG	TTATGTTGCA	AATACGCTG	ATCCATCCGT
35281	TGTAAGGCTA	ATTTCAGATG	TTCTCCGACC	AGCAGCCCCT	GATAAAGATC	ATTCCAGAGA
35341	CCACTTTGGA	CGAAATTCAT	ATCATACTGA	CCTGTTTCGT	ACTGCCAGGA	GGCTTCGGCC
35401	AGTAAACAGA	GGGAATTAA	CGCATCATAG	GCTTGCAAGT	AAAGCCGGAG	ATTTGGCTGA
35461	TCATCCACAT	GTATAACGCA	TCATTGGTAN	ANTTGTTCNN	NNNNNNNNNN	NNNNNNNNNC
35521	CCGAAGCATA	CCGCCAAGAC	CATCCCCCGG	ACGGCCAGAC	CGAAATATTT	GGGAACCCATA
35581	TCCGCCACAG	CGGCCGCGAG	GGCGGCTGAC	TGGGCAGCGA	TCACACCTTC	AGCCGCTCTT
35641	GATTGTAATG	CGATAACTTC	CTGCTCGGTG	ATGGAGATGT	TTTCATCATG	GAGCGATTTA
35701	TAGTGTGTCT	GGCGCTCCTG	AGCGGCCCGT	CGGCTGATGG	TCAGTGCATC	CAATGAAGCC
35761	TGTTGCATGT	CAATCGCTTG	CTGTTGCAGA	TTGCGGGTAA	AGCTGTACAG	CCCCAGTTGC
35821	TGCTGCATAC	GGAAAGTGTTC	AAAATCGGTA	TTGTCTTTTT	TCTCCAGCAA	CCTCAGTAAC
35881	GTGCTGCCGT	ACTGAATCAG	CGTTTCTGCG	GCCTCTTTTG	CCCGGCTCAT	GATCGGGGTG
35941	AAACGATAAT	TCGGGATTGC	CCGGCGTTTC	ATGCCCGCCA	TACGATTAGC	CACAACACGC
36001	TGGTAACGCT	GCCTGAGCAG	ATCTTGCGGG	CTGATGGGTT	CATCGTATAA	TCCGGCCCGGA
36061	AACTCTTTAC	CATCCAAGGT	CAGGTTATGA	CGTAAGTTAT	ATAGACGCTG	ATCCAACATT
36121	TGCCACAGTT	TGAGATATTC	CGTATCAACA	GGTTTGACAA	ATAAATCAGA	CGGTGCGGCA
36181	GAGACGGATG	TATCATATGT	CACAGGCAGA	AGTGGCACGT	TGCTGACAGT	AAGCATTAA
36241	TCCTGTGCCC	GTGCTTCACT	GTTTTCATAC	AGAGCCACAT	CTTGACGCGT	ACGGGGTTGC
36301	CAGTTTGCCC	CGAGCAGAA	ATCAGGGCTG	GTACCCAGTA	ACATATTGAC	AGGATCATAG
36361	ATCTGCTTGG	CGACAGTACG	TGCACTGGAT	GTCAGCTTAC	GGTATTCCAT	GTCTCCCTGA
36421	TCTAACAGAT	TCTTGACATA	GAAACGGAAT	ATTGCTTTCC	GGTAGTGAAT	GGGTTCACTG
36481	GCTGCAATGG	CATCCGGATC	GGTTGGTTCA	ATTAACATCC	GGTACACGGT	GGGTGGAGGA
36541	TCAATAATTG	GCCGTGAATT	CCAGTAACGC	GGTTTACCTT	GGTTGCTGGC	CTGAACAGT
36601	TCATCTTCCA	GCGGATTAAA	AATATAGTGC	AGCCATTCCG	TGGCCTCTTT	TAATCGTTGT
36661	TCTATATTCA	GTGCCACGCG	GACCAGAAAT	GGCATATGGA	AAAACAGTTC	CCAGAAATAG
36721	ATCCCATTTG	CGCCATTTAA	ATCAATCGGC	GTAGGGAATG	AACCGGGTAT	AGGCTGTTCC
36781	GTAATAAGCT	GTGTATTCCA	GCTCAGTACC	TGCGGGATAC	CCTGACTGGC	AATGGCGATC
36841	AGTTTTTTTG	CAAACAGTGT	ATTAAGGCGA	ATGTTTTGTG	GCGCGTTATC	AGTTTTCATC
36901	GCGGGGAAGG	AAAGGAATTG	CACCTGATCC	TGTTTCATTGA	GTTTAAATCAG	TTCCGGAATA
36961	TGCATACCGA	TTCTGAACTC	TTGAGTACAG	CTGGCACTTT	CATTGCCAAC	ACCACCTTTG
37021	GGCTTAAAGA	GAAGTTCGCG	TTTCAGGGTG	ATTGCAATTAT	CCGACCCGAC	CTTGATTGAT
37081	GGATAGGTTA	AATCAAGAAC	TTTTTCGCTC	AGTACCAAGT	GTTGTTTCATC	CAAGACAGTA
37141	TTATCGTGCA	TCAGCCGGAA	AGAACCCTTG	TAATATTGAT	GATCTTCTAT	CGCACCAGAA
37201	TTAAAGTCAG	ATTGAGCGAC	AATCTCCAGT	GTGTCATCAG	TGCCATGAAC	AAAATTGACA
37261	ATCAGTTTGA	TACTGTCTTT	GCCGAAATCA	GGGTTTCAATC	CGGTTTGGAT	TCTCCGGCAA
37321	TAGGAAAGCG	TTCTTCCCGG	GTTGCCGGAT	AGAGCACCAT	AGTACGGTAA	TCGATAGGAT
37381	TGCCTTAAGG	CATCCTTGTG	TTACAGTGAG	TAATACCAGA	CCAGGTTGCC	GACATATTTT
37441	CCTTTTCGTC	CATCAGCATA	TTGGTCATCC	GGCAAATCAG	TAATTTCTAC	CAGCAGTGTA
37501	TCGACAGCAT	AACCGAAGGC	TTGCTCATAA	TCATAATCCT	TACCTTTCTT	ATCTGTCCCC
37561	TGAAGACGGA	CAAACGGAAC	CAGAGCCAGA	AACGGGTTAT	GCGGGTCTTG	CTGTATATCC
37621	ATCACAGCAA	CCATCTGGGC	CATCCGGTAT	TGCAGATGTC	TTCCGCGAGA	ATGGTGGGTG
37681	TACTCCAGCT	GCCATCATAT	TTGGCATAAG	CGATTTTGAT	CCGGTCAGGA	ACGGTGTGGG
37741	AGGAACCCAA	TCACCCGCA	TAGGCTCAAC	GTTTTGGTTA	TGCAGTGATA	ACGCAGTTGT
37801	ATCTTTAGTT	TCAGACTGTT	CTTCAACTTC	CGTCCAGGCA	ATATACAGGC	GATTATTCAG
37861	GAAAATGGGG	CGTATCAAAT	TGGGGTCTAC	GCTGCCCAAT	GGCAGGTCAA	TAGGTTTCCA
37921	CTCGCTCCAG	GCATTGGGAG	ATAACGCATC	GGTATCAGGA	TGGCGTATCG	AAAGATTTCAG
37981	TGAACGCCAG	TAATATTGGT	ATGGCTGTGT	ACGGGTACGT	CCGACAAAGA	AGAACTTATC
38041	GCGTTTGATG	TTAACACCAT	CTTCATAACC	TGCGATAACT	TTTCAAGTTAC	TGACATCTTC

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Fig.2.

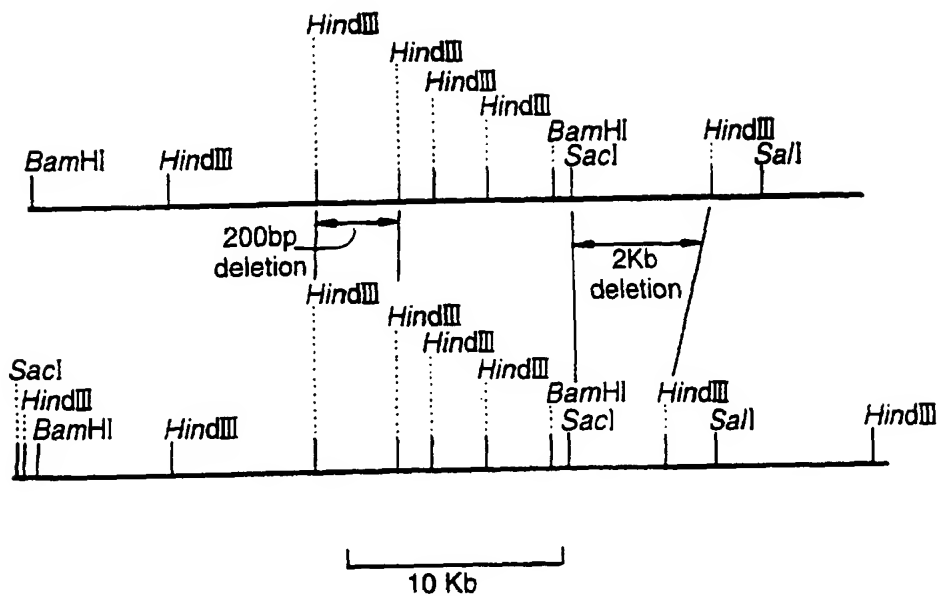
```

38101 AAAATTATTC AGATAACCGA GCACCGCTTG TTGTACAGAA TCTTCGGTAA TTTTCCCTG
38161 ATTAAGGGCA CTTTCAGTT GGAAGAAGAA TTCTGTTTAA TTCAGGCGTA ACAGGGGTTT
38221 CAGATAGCTT TCCGATAAG TCCGTAATAA GCGATCCC

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N=unspecified base

Fig.3.



INTERNATIONAL SEARCH REPORT

national Application No

PCT/GB 97/02284

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A01N63/02 A01N63/00 C12N1/20 C07K14/24 //(A01N63/02, 63:02,63:00),(A01N63/00,63:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 00647 A (COMMW SCIENT IND RES ORG ; SMIGIELSKI ADAM JOSEPH (AU); AKHURST RAY) 5 January 1995 cited in the application	1,5,11, 13, 18-21, 24-26, 29,30,32
Y	see page 1, line 3 - line 29; claims 10-13	3,4, 6-10,12, 14,27, 28,31
	---	-/--

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

17 December 1997

Date of mailing of the international search report

14/01/1998

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Muellners, W

INTERNATIONAL SEARCH REPORT

national Application No
PCT/GB 97/02284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>CHEMICAL ABSTRACTS, vol. 118, no. 1, 4 January 1993 Columbus, Ohio, US; abstract no. 3550, YAMANAKA, SATOSHI ET AL: "Biochemical and physiological characteristics of Xenorhabdus species, symbiotically associated with entomopathogenic nematodes including Steinernema kushidai and their pathogenicity against Spodoptera litura (Lepidoptera: Noctuidae)" XP002048914 see abstract & ARCH. MICROBIOL. (1992), 158(6), 387-93 CODEN: AMICW;ISSN: 0302-8933, 1992,</p> <p>---</p>	3,6
Y	<p>DATABASE DISSABS STN-International / UMI Company STN-AN 96:33246, DISSABS order no. AAI9608671 , 1995 DAVID JOSEPH BOWEN : "Characterization of a High Molecular Weight Insecticidal Protein Complex Produced by the Entomopathogenic Bacterium Photorhabdus luminescens (Nematodes, Biological Control)" XP002048915 see abstract & DISSERTATION ABSTRACTS JOURNAL INTERNATIONAL , vol. 57, no. 18, 1995, page 93</p> <p>---</p>	4,12,14
Y	<p>EP 0 238 441 A (CIBA GEIGY AG) 23 September 1987 see page 1 - page 2 see page 4, paragraph 3 - page 5, paragraph 2; claims 10,12,22,36,37</p> <p>---</p>	7-10,27, 28,31
X	<p>WO 84 01775 A (COMMW SCIENT IND RES ORG ;BIOTECH AUSTRALIA PTY LTD (AU)) 10 May 1984 cited in the application see page 1 - page 3, line 10 see page 4, line 24 - line 28 see page 4, line 36 - page 5, line 3 see page 14, line 17 - line 29 see claims 26,27</p> <p>---</p> <p style="text-align: center;">-/--</p>	1,4,5, 11,13

INTERNATIONAL SEARCH REPORT

national Application No

PCT/GB 97/02284

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H.MATSUI ET AL. : "Nucleotide sequences of genes encoding 32 kDa and 70 kDa polypeptides in mba region of the virulence plasmid, pKDSC50, of Salmonella choleraesuis " NUCLEIC ACIDS RESEARCH , vol. 18, no. 8, 1990, pages 2181-2, XP002050055 see the whole document ---	21-25
X	F.BINDER ET AL.: "Cyclodextrin-glycosyltransferase from Klebsiella pneumoniae M5a1: cloning nucleotide sequence and expression" GENE, vol. 47, 1986, pages 269-77, XP002050056 see page 269, the summary see page 270, right-hand column, last paragraph - page 271, right-hand column, paragraph 1 see fig. 3 bp 2641-2809 ---	21-25
P,X	US 5 616 318 A (DUDNEY RALPH A) 1 April 1997 see column 1, line 65 - column 2, line 52 see column 5, line 3 - line 4 ---	1,4-6, 11,13
T	WO 97 17432 A (WISCONSIN ALUMNI RES FOUND) 15 May 1997 see page 2, line 31 - page 3, line 23 see page 5, line 1 - line 16 see page 8, line 23 - line 33 see page 9, line 41 - page 11, line 14 see page 17, line 1 - line 21 -----	1-32

INTERNATIONAL SEARCH REPORT

national Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EP 0238441 A	23-09-87	GB 2188049 A AU 608508 B AU 6999287 A BG 46006 A BG 46752 A BR 8701162 A DE 3788077 D DK 128687 A EG 18869 A ES 2059404 T IE 59456 B JP 62224295 A	23-09-87 11-04-91 17-09-87 15-09-89 15-02-90 12-01-88 16-12-93 16-09-87 28-02-94 16-11-94 23-02-94 02-10-87
WO 8401775 A	10-05-84	AU 558287 B CA 1214130 A EP 0126092 A US 4672130 A	22-01-87 18-11-86 28-11-84 09-06-87
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WO 9717432 A	15-05-97	AU 1050997 A CA 2209659 A EP 0797659 A	29-05-97 15-05-97 01-10-97

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁶ : A01N 63/02, 63/00, C12N 1/20, C07K 14/24 // (A01N 63/02, 63:02, 63:00) (A01N 63/00, 63:00)</p>	<p>A1</p>	<p>(11) International Publication Number: WO 98/08388 (43) International Publication Date: -5-March 1998 (05.03.98)</p>
<p>(21) International Application Number: PCT/GB97/02284 (22) International Filing Date: 27 August 1997 (27.08.97) (30) Priority Data: 9618083.1 29 August 1996 (29.08.96) GB (71) Applicant (for all designated States except US): THE MINISTER OF AGRICULTURE FISHERIES & FOOD IN HER BRITANNIC MAJESTY'S GOVERNMENT OF THE UNITED KINGDOM OF GREAT BRITAIN & NORTHERN IRELAND [GB/GB]; Whitehall Place, London SW1A 2HH (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): JARRETT, Paul [GB/GB]; 14 Home Furlong, Wellesbourne, Warwickshire CV35 9TW (GB). ELLIS, Deborah, June [GB/GB]; 7 Cooke Close, Warwick, Warwickshire CV34 5YG (GB). MORGAN, James, Alun, Wynne [GB/GB]; Pen-Y-Goruf Farm, Gorof Road, Ystradgynlais, Swansea SA9 1TP (GB). (74) Agent: SKELTON, S., R.; D/IPR, Formalities Section (Procurement Executive), Poplar 2, MOD Abbey Wood #19, P.O. Box 702, Bristol BS12 7DU (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>
<p>(54) Title: PESTICIDAL AGENTS</p> <p>(57) Abstract</p> <p>A method for killing pests (e.g. insects) comprising administering material from <i>Xenorhabdus</i> species (e.g. <i>X. nematophilus</i>) such as cells or supernatants orally to the pests, either alone or in conjunction with <i>Bacillus thuringiensis</i> or pesticidal materials derived therefrom. Also disclosed is an isolated pesticidal agent (and compositions comprising the same) characterised in that it is obtainable from cultures of <i>X. nematophilus</i> or mutants thereof, has oral pesticidal activity against <i>Pieris brassicae</i>, <i>Pieris rapae</i> and <i>Plutella xylostella</i>, is substantially heat stable to 55 °C, is proteinaceous, acts synergistically with <i>B. thuringiensis</i> cells as an oral pesticide and is substantially resistant to proteolysis by trypsin and proteinase K. DNA encoding pesticidal activity is also disclosed.</p>		

* (Referred to in PCT Gazette No. 29/1999, Section II)

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